WATER CHEMISTRY AND PHYTOPLANKTON FIELD AND LABORATORY PROCEDURES

Curtiss O. Davis
and
Mila S. Simmons
Editors

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Great Lakes Research Division
The University of Michigan
Ann Arbor, Michigan 48109

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TABLE OF CONTENTS

I. PREFACE

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I. PREFACE

This manual was written to serve two basic purposes: 1) as a guide for persons using these techniques in water quality studies, and 2) as a written record of the methods used in this laboratory at this time. It is anticipated that the manual will be updated frequently as new methods are added and the present ones are further refined. The present methods are all used routinely and have been in regular use for a year or longer.

This manual is specifically written as a guide for the collection and analysis of lake water samples from the Laurentian Great Lakes. However, all of the analytical methods are easily adapted for laboratory culture or small lake studies. The descriptions contained in this manual are designed primarily as users guides oriented to the equipment available at the Great Lakes Research Division, and as most of the methods are taken from the literature, the reader is referred to the original articles for a more detailed discussion of the methods.

Sections of this manual were written by the staff of the Nutrient Chemistry and Phytoplankton Laboratories of the Great Lakes Research Division. Field Procedures were prepared by Curtiss Davis, Hung K. Soo, and Mark Weishan; Laboratory Procedures, General by Mark Brahce, and Soluble Nutrients by Curtiss Davis, Douglas Scales, Harold Davis, and Jerry Krausse; Particulates by Mila Simmons, Jill Goodell, Jerry Krausse, and Mary Hui; and Phytoplankton Parameters by Eugene Stoermer, Russell Moll, Laurie Feldt, Thomas Berry, and Paul Friedrich; with drawings by Andrée Valley. We wish to acknowledge Dr. Claire L. Schelske's support and valuable comments during the preparation of the manuscript.

II. FIELD PROCEDURES

Introduction

When the ship arrives at a station all scientific personnel should appear on deck to help with the tasks on that station. Each person is assigned a specific task and is responsible for preparing equipment, sample bottles, etc. needed to do that task efficiently upon the ship's arrival at the station. This section outlines the general procedures to efficiently complete a typical water chemistry and phytoplankton sampling at a station.

Equipment and Supplies

Log book and blank log sheets (Fig. II.1.1)
black ink ballpoint pens
other equipment as described in the section for the particular samples to be collected

Procedure

The specific activities will vary from cruise to cruise, but in general the following will be done on each station: One person is responsible for filling out the top of the log sheet (Fig. II.1.1), taking surface temperature, Secchi disk and, with an assistant, quantum meter readings. A second person, assisted by the winch operator, uses a BT winch to lower the BT and take all necessary water bottle samples. The person assisting with the quantum meter generally assists with handling the full water bottles. A third person may be taking net tows or other samples from a second BT winch. Persons responsible for water filtration, pH, conductivity, ^{14}C, etc. should start processing samples as soon as possible after the water bottles are returned to the rack. It is important that all samples be processed and properly stored as quickly as possible.

The person in charge of each station should fill out as much of the log sheet (Fig. II.1.1) as possible before the beginning of the station. This includes: year, lake, project, investigator, cruise, date, depths to be sampled, and sample numbers at those depths. The rest of the top of the log sheet is filled out during the station as mentioned previously.

The remainder of the log sheet, from temperature down, is filled out by the person involved with each parameter, as necessary. Temperature (this is the temperature measured while taking the pH and should not be confused with the *in situ* temperature which is read from the BT.), pH, conductivity, and alkalinity values are recorded by the person making these measurements. The BT slides should be read and values for the sample depths recorded as soon as possible. The rest of the spaces are reserved for special comments such as; only 525 mL filtered for total silica at 10 m, what depths were sampled for C-H-N, etc. Any abnormalities should be recorded on the log sheet immediately by the person observing the abnormality.

YEAR_____ LAKE_____ PROJECT_____ INVESTIGATOR_____

CRUISE_____ DATE_____ STATION_____ TIME_____ _____
on sta. off sta.

WHITE SECCHI_____ SURFACE TEMP._____ COLOR_____

SEA STATE_____ SKY CONDITION_____ AIR TEMP._____

B.T. #_____ SONIC DEPTH_____

Niskin Bottle

Depth															
Sample No.															
Temperature															
pH Value															
Conductivity Value $x10^{-4}$															
Alkalinity															
Chlorophyll 250 ml-HA															
Raw Water															
Soluble Chem.															
Total Silica 600 ml-HA															
GFC C/N															
Gross Phyto 1000 ml-AA															
Phytoplankton															
BT Reading															

Screen

TIME IN:_____ TIME OUT:_____

0	Vial #														
	Tray #														
1	Vial #														
	Tray #														
2	Vial #														
	Tray #														
Dark	Vial #														
	Tray #														

General Comments:

FIG. II.1.1 Station log sheet.

Introduction

The first step in analyzing water quality characteristics of a particular part of a lake is to obtain an uncontaminated water sample from the depth and location of interest. Discrete water samples are collected using Niskin water bottles suspended to the appropriate depth on a weighted "hydro" wire.

Principle of the Method

Niskin bottles are nontoxic PVC water samplers. They are attached to a weighted wire and lowered with both ends open to the desired depth. A messenger, a brass weight which fits around the wire, is then dropped, tripping the closing mechanism and thereby capturing a discrete parcel of water from the desired depth.

Equipment

Niskin water samplers (usually 5 L)
messengers
modified bottom tripping Niskin bottle
bottom tripping weight

Procedure

Water samples are taken with either 5 L or 8 L Niskin bottles. Bottles are used starting from the right side of the rack, i.e. if 5 out of 12 bottles are used, the first 5 bottles are taken starting from your right as you face the rack. Check that everyone is finished using the water in these bottles from the previous station before taking them. The person hanging the bottles on the wire must wear a working life vest while on the "Hero board." The bottles are attached at intervals on the wire according to a winch card prepared as follows:

1. Desired sample depth (meters)	2. Greatest depth - sample depth	3. Invert 2 = winch card
1	59	0
5	55	20
10	50	40
20	40	50
40	20	55
60	0	59/1

The winch operator will zero his meter and the first bottle is attached. The winch operator then lets out 20 m of wire and the second bottle is attached. The wire is let out to 40 m and the third bottle is attached, etc. The /1 means that after the last bottle is attached it will be lowered to 1 m below the surface. A messenger is attached to each bottle except the bottom one, and is clamped around the wire below the bottle. When the bottles are lowered to the correct depth a messenger is dropped down the wire to trip the bottles. Lightly hold the winch wire between the thumb and fingers and count the bottles as they trip. Be certain that all the bottles have tripped before signaling the winch operator to raise the bottles. If there is any question that the bottles did not trip properly the cast should be repeated to obtain a proper sample.

At some stations a bottom

tripping bottle is used along with the regular bottles. A special weight is used with a cup on top to hold the 1 m tripping arm on the bottom tripping bottle. The bottom tripping bottle is mounted upside down on the wire with the tip of the tripping arm inside the cup, but not touching the bottom of the cup. Do not hang a messenger on the bottom tripping bottle, or the bottle above it. After the other bottles have tripped, the winch operator will let out more wire until the weight touches the bottom. The bottom of the cup will then touch the tripping arm, closing the bottom tripping bottle 1 m above the bottom. When the bottom tripping bottle is to be used a B is marked on the first line of the winch card, above the 0.

After all the bottles are tripped the winch operator raises the bottles. They are removed one at a time and placed in the rack with the first bottle (nearest to the surface) going in the right hand space as you face the rack. Any variation from this routine must be clearly marked with tape on the rack, and explained to everyone using water from that station.

Introduction

Many chemical and biological parameters change rapidly once a sample of water has been removed from its natural environment. For this reason water samples should be processed and preserved immediately upon collection. This section describes the initial treatment of samples to prevent deterioration of sample quality for the basic types of phytoplankton and nutrient samples described in the other sections of this manual. Frequently individuals not involved in the sample analysis are involved in the initial shipboard operations, and this section is designed as an overall guide for those initial sampling operations.

Procedure

Water samples are processed according to a flow diagram similar to that in Fig. II.3.1. The amounts of water filtered and particular analyses to be done may vary from cruise to cruise, and a proper flow diagram for that cruise should be posted near the filtering apparatus. There are typically four types of filtered samples (Fig. II.3.1). Gross phytoplankton samples are filtered using AA Millipore filters. They are stored in amber vials containing Transeau's solution (6:3:1) as preservative.

C-H-N samples are filtered using glass fiber (GFC) filters. The filters are folded in half with the sample on the inside, stored in glassine envelopes, and placed in a heated vacuum desiccator at 60°C with the valve slightly open to allow a very slow flow of air over the samples. The envelopes containing the samples from each station are clipped together with a paper clip and placed in the desiccator with the envelope opening up so that when the filters dry they do not fall out of their envelopes. When properly handled, a full day's set of filters will be completely dry overnight. Each morning the filters should be removed from the vacuum desiccator and placed in a coffee can layered with silica gel at the bottom. The samples are kept dry in the freezer until analyzed.

Chlorophyll a samples are filtered using HA Millipore filters. The filters containing the residue are extracted in 90% buffered acetone in amber vials. These vials are stored in the freezer as quickly as possible. The samples should never be exposed to direct light.

Particulate silica samples are collected on presoaked Millipore filters and are stored in fliptop plastic vials in the freezer until analyzed. Water filtered for the particulate silica samples is collected in two 1-oz widemouth linear polyethylene bottles for nutrient analysis. If not analyzed that day they should be frozen for preservation (Note 3.3).

Raw water samples for particulate phosphate analysis are stored in 2-oz polyethylene bottles in the freezer. The initial sampling for the other analyses shown in Fig. II.3.1. should always be done by the person conducting those analyses, and therefore, the reader is referred to the appropriate sections of the manual for those procedures.

Notes

1. It is important to do all the filtering work quickly and careful-

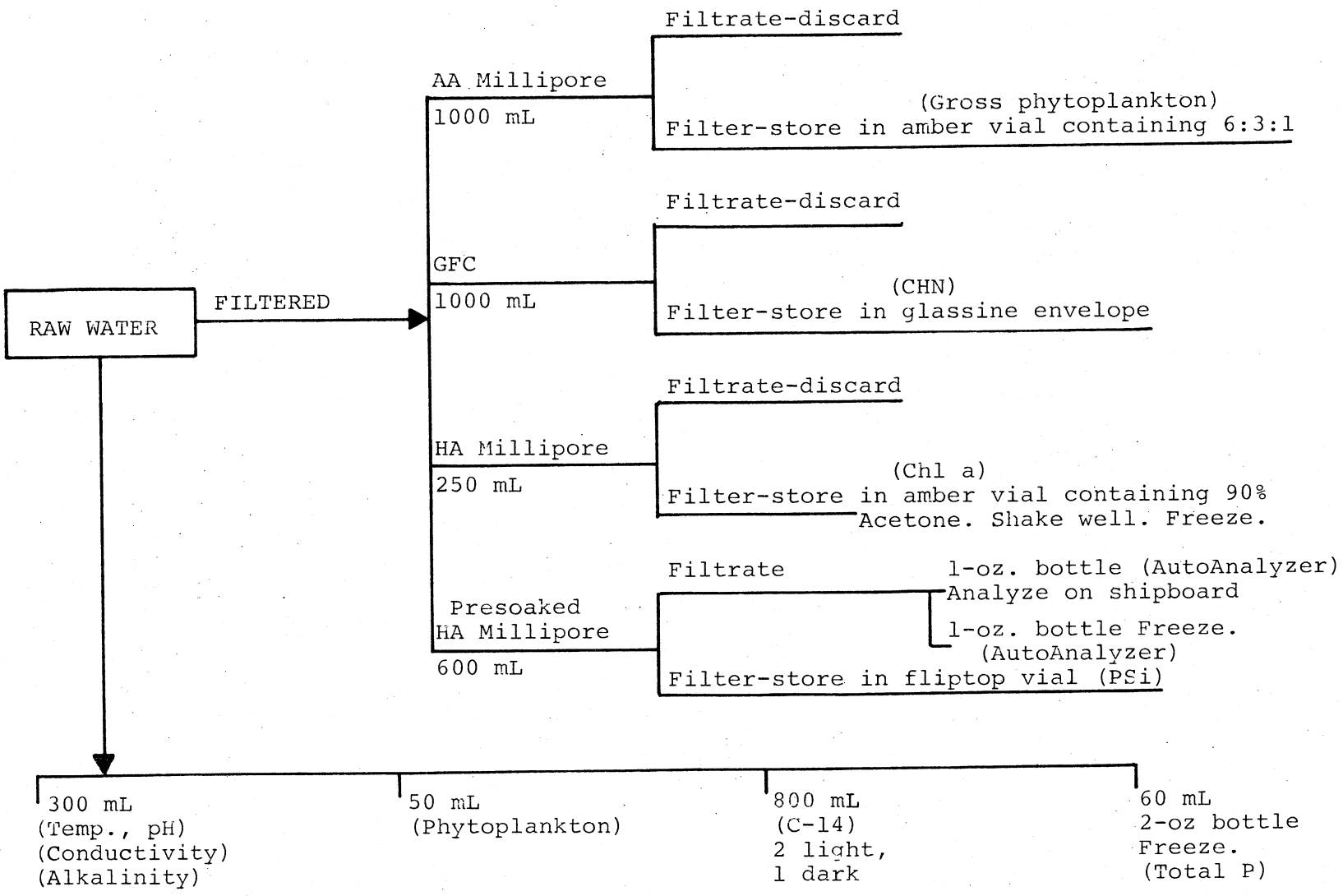

FIG. II.3.1 Water chemistry filtering scheme. ( ) indicates the eventual use of that subsample.

ly, and to store the samples properly as soon as possible. Care must be taken to measure volumes accurately, never to touch filters (use forceps) or sample water, to keep all equipment clean, and to be sure the right sample is in the right bottle.

2. If the Niskin water bottle has been sitting in the rack for 30 min or longer remove the bottle from the rack and shake it before taking a sample.

3. Water samples to be frozen should be filled to no more than the bottom of the neck of the bottle to allow room for expansion during freezing.

4. Any deviations from the norm should be recorded immediately on the log sheet.

Introduction

The mechanical bathythermograph (BT) is an instrument for obtaining a graphic record of water temperature vs. depth. The record is scratched by a stylus into a thin gold coating on a glass slide. The record is obtained by lowering the instrument through the water column. The data are read from the slide by viewing it through a calibrated grid, in a special grid holder with an integral magnifier.

Equipment and Supplies

BT calibrated for appropriate depth range (0-61 m, 0-152 m or 0-305 m)
BT slides
slide reader with grid calibrated for BT used
clear acrylic spray paint

Procedure

To put the slide into the BT, first check that the grooves in the slide holder are clean and free from glass chips. Handle the slide by its edges. Insert the slide into the slide holder, bevelled corner first, with the bevel (or longer bevel) toward the nose of the instrument. This ensures that the coated surface will be against the stylus. Check that the spring holds the slide firmly against the top groove, and that the slide is pushed all the way in against the stop pin.

After the depth of the water has been determined, and permission has been obtained from the bridge, signal the winch operator to start the cast. Pick up the BT and slide the stylus lifter sleeve toward the tail to cover the slide holder. Hold the BT over the rail until the winch operator takes up the slack with the winch, then allow the BT to hang free over the side. The winch operator will lower the BT to just below the surface and stop for one minute to allow the temperature sensor to equilibrate before lowering it further. At this time take a bucket sample to measure the surface water temperature. Collect a container of surface water, and immediately measure its temperature with an accurate thermometer. Immerse the bulb end at least 3", stir with the thermometer for 15-20 seconds, and then read the thermometer with the bulb still immersed. Record the temperature on the log sheet.

The winch operator will lower the BT to near the bottom or to the maximum depth for that BT, whichever is less, and then haul it back to the surface. As soon as the BT is in hand slide the stylus lifter sleeve forward to prevent extraneous scratching of the slide. On deck partially eject the slide by pushing it with your finger or the eraser end of a pencil through the slide ejector port. Grip the slide by the edges with the thumb and forefinger, remove it from the BT, and place it in a slide box until it can be annotated and coated. If another BT cast is to be made soon, lash the BT securely to the rail. Otherwise, unshackle the BT and secure the bitter end of the wire. Secure the BT in its rack or packing box.

Slide Preparation

When the slide is dry, use a pencil or other pointed instrument to scratch the following information into the gold coating, in a place where it does not interfere

with the trace: BT serial number (stamped on the nose of the BT. Prefix this with the letters 'BT', e.g. BT 5005), date (da/mo/yr), ship name and cruise number (or use a letter designation for the ship, L-115 would be LAURENTIAN cruise 115), and station number or consecutive lowering number.

Coat the slide by either of two methods. Hold the slide by a corner with tongs. Spray a light, even coat of clear acrylic spray over the gold covered surface of the slide. Hold the slide until dry, about one minute. This method is easiest, and is preferred. The slide may also be dipped in thin lacquer, and the excess allowed to run off. The slide is then placed in its box to dry, and lifted when the drying is nearly completed, to prevent sticking.

Reading and Interpreting the BT Slide

Insert the slide into the viewer with the gold-coated side toward the grid. Make sure the grooves are clean, that the slide is seated firmly against the stop pin, and that the spring holds the slide firmly against the grid. Look through the magnifier toward a white reflector or window, and adjust the focus. Adjust the stop pin with a screwdriver so that the surface temperature agrees with the bucket surface temperature. Check that the grid is the correct one for the BT used for making the slide. The temperature/depth profile is read where the scribed trace on the slide intersects the lines of the grid. The curved horizontal lines are depth, and the tilted vertical lines are temperature. Temperature may be read to 0.1°, and depth may be read to a tolerance of 1% of the total depth. Read from the center of the scribed line. Record the BT temperature for each bottle sample depth on the station log sheet, plus any special comments as "sharp thermocline at 15 m," or "2 traces, read left one," etc.

ABNORMALITIES - If two traces are seen (hysteresis) a malfunction is indicated. If there are sharp spikes at the bottom of the trace, or along the trace, the BT hit the bottom, or an object in the water. If there is no trace, the stylus lifter sleeve may not have been slid back, or a malfunction may be indicated.

Notes

1. The accuracy of the BT is dependent on careful handling. Care must be taken to protect it from jars, shocks, and extremes of temperature. If the BT is dropped, banged against the side of the ship, run into the bottom, or subjected to any severe shock, its calibration is suspect from that point on, and another instrument should be used if available.

2. The temperature sensor will jam into its stops and be bent if the temperature is allowed to exceed $105^{\circ}F$ or drop below $20^{\circ}F$. Never leave the BT on deck in the sun during summer. The instrument may be covered with a wet cloth to protect it. Never leave the BT in an unheated space in freezing weather. The pressure (depth) sensor will be damaged if the BT is lowered past its maximum depth.

3. The gold coating on the slides is thin and easily rubbed off or fingerprinted. The slides should

be handled by their edges only, and they should be coated with lacquer or acrylic as soon as possible after removal from the instrument, preferably before any data are read from them.

4. Because of slight differences in the mechanical pressure and temperature sensors in each instrument, the grid for each instrument is unique. The slides made using a particular instrument must be read with the grid prepared for that instrument, or the data obtained will not be valid.

References

U.S. Hydrographic Office publication #H0607 Instruction Manual for Oceanographic Observations

Wallace and Tiernan Inc. Instruction book #FIA 190-1-1 Instructions For Installation, Operation, and Maintenance of Wallace & Tiernan Apparatus

Dittmore - Freimuth Corp. Instruction Manual- Bathythermograph

Introduction

The YSI Tele-thermometer is a thermistor probe system with a Rustrak recorder for making continuous temperature records. It is usually used to make records of surface temperature while the ship is underway in one of two modes: a) a weighted thermistor is towed alongside the ship at approximately 1 m depth, or b) water from 1 m depth is pumped into the laboratory from the ship's sea chest system and the thermistor is placed in the flow immediately as it enters the laboratory (usually in the debubbler for the underway mapping system). Operation of the thermistor probe is simple, and reliable data are obtained if the following directions for maintenance and calibration of the thermistor and recorder are followed.

Equipment and Supplies

YSI Tele-thermometer unit
thermistor probes
Rustrak recorder unit
chart paper for Rustrak recorder
spare batteries

Calibration and Maintenance of YSI Tele-thermometer

Open the indicator panel by loosening the four knurled screws. Check that the battery terminals are free from corrosion. If corrosion is present, clean terminals and replace battery immediately. Check also that the contacts of the probe receptacle are clean.

Replace panel and observe that the meter is reading "50" with the power off. If necessary, adjust the meter to read "50" by turning the slotted pin on the meter directly above the pivot of the meter needle.

Turn the power on, and with nothing connected to the probe receptacle adjust the slotted brass shaft (right side of the meter) until the meter needle is on the red line (5°C). If the needle cannot be adjusted beyond the red line, (i.e. it reads below 5°C) replace the battery.

Plug in a YSI thermistor probe and check the reading with a mercury thermometer at 0°C (ice bath) and approximately 20°C in a well-stirred, insulated water bath. If the readings do not agree, it may indicate a bad probe or bad contacts. A broken probe will cause a high reading and a shorted probe will cause a low reading. If a second probe gives identical readings to the first then the probes are most likely functioning properly and the thermistor bridge should be readjusted.

To adjust the thermistor bridge remove the indicator panel and adjust the red line rheostat and the two internal rheostats in the following sequence: 1) with the thermistor which is at 0°C connected to the meter, adjust the meter to read 0°C with the red line rheostat (hold the meter horizontal for most accurate readings); 2) switch to the probe in the room temperature water bath, and adjust the rheostat next to the + battery terminal so that the meter reads the correct temperature; 3) unplug the probe and adjust the meter to the red line with the rheostat next to the - battery terminal. Repeat 1, 2, and 3 until the values converge. Replace the indicator panel.

Turn on the power and let the recorder warm up 15-20 minutes.

Turn the chart drive on. You will notice that the striker plate starts to move. Turn the chart drive off immediately after the striker plate strikes so that the needle on the recorder is free to move. Set the recorder range to the 10 MV position. Connect the YSI Tele-thermometer outputs to recorder inputs with the red output connected to the LO input and also tied to GND. The black output is connected to the HI input of the recorder.

To calibrate the recorder, turn on the thermistor and dip the probe in a bucket of water at about 20°C, then make the following adjustments:

a) Unplug the probe so that the meter reads 5°C (see 3 above) and adjust the zero offset on the recorder until the recorder indicates 20% deflection (0-25°C full scale). Use the intersection of the edge of the striker plate and the needle as reference. The needle moves left to right with clockwise rotation of the zero adjustment. If the needle will not come on scale using the zero adjustment (eight turns is full range; after that the pin spins but does not change the reading), bring the needle on scale with the span, then adjust to 20% deflection with the zero adjustment.

b) Plug in the probe and read the correct temperature. The recorder should agree with the thermistor reading. Adjust it to the proper reading with the zero adjustment.

c) Detach the probe and adjust the recorder to 20% deflection using the span.

d) Repeat b) and c) until the readings converge on the true values. If the recorder was badly out of adjustment to begin with; the process will proceed more quickly if the reading is overadjusted by 10-20% for the first few times. Accurate settings are usually achieved after 6 or 7 repetitions.

Notes

1. When the meters are old and worn one may need to tap the meter slightly to overcome friction. DO NOT tap on the glass, tap only on the bakelite. The battery is not efficient in cold weather, and sub-zero weather also slows meter movements. It is best to keep the meter in the ship's cabin and run extensions for the thermistor probes.

2. The calibration is done for the range 5 to 20°C. This is the normal working range, and the thermistor is linear over that range. As indicated by the meter scale, it is not linear from 0 to 5°C. When working primarily in that range the recorder should be calibrated using an ice bath and the 5°C setting.

Introduction

The Eppley pyronometer is used to measure daily incident radiation in langlies/min. These data are used in conjunction with the underwater light profile measurements (sections II.6.2 and II.6.3 to obtain an estimate of the light for photosynthesis available to phytoplankton growing in the lake.

Equipment and Supplies

Eppley pyronometer (mounted on gimbals with 50' cable)
Rustrak recorder unit
Rustrak chart paper
spare batteries (mercury cell #RM 625 1.3V)

Procedure

The Eppley pyronometer is mounted on gimbals so that it will always point upward regardless of the ship's roll. It should be securely attached by the lower gimbal to a location on the ship which is exposed to the direct sun and has a minimal potential for shadows of the ship's mast, etc., falling across it. The wires should be taped down to keep them off the deck and out of the way. Care must be taken that there is ample free wire at the base of the pyronometer so that it can pivot freely in all directions and maintain its vertical position when the ship rolls. From the pyronometer the wire is led into the laboratory and attached to the pyronometer Rustrak recorder unit.

Calibration of the YSI Eppley Pyronometer Recorder

The Eppley pyronometer has a fixed calibration; however, to obtain an accurate record the recorder should be calibrated before each cruise. The Eppley pyronometer has a calibration stamped on its name plate (e.g. 6.90 mv/langley). Use the Honeywell "Rubicon" portable potentiometer as a reference to set the span for the recorder corresponding to the calibration given for the particular pyronometer.

Put batteries in the potentiometer and replace the potentiometer in its carrier. Set all dials to zero and the Function switch to SC (standard cell). Push the button "GA" momentarily and adjust "STD" knob until a null is obtained (i.e. galvanometer has no deflection when the "GA" button is depressed). Note - do not hold down the GA button for extended periods or the internal standard cell will be ruined.

Set the function switch on the potentiometer to output 0.1. The sum of the two dial readings x 0.1 will give you the reference voltage you require at the output EMF posts.

Connect the + EMF to the Hi terminal of the recorder and - EMF to the Lo terminal of the recorder. Turn the recorder on with the chart drive off at a point in its cycle when the needle is free from the striker plate. Set the range to 10 mv and adjust the zero offset to give a zero reading on the recorder with the Honeywell potentiometer dials set to zero. (Note both step switch [0-160] and millivolt knob must be on zero).

Set the Honeywell potentiometer output to the required EMF specified by the pyronometer (e.g. 6.9 mv/langley - then set pot to 13.8 mv) and adjust the span to full scale indication on recorder. The recorder is now adjusted to record 2 langleys full scale which

is the proper range when used in full sunlight.

Disconnect the Honeywell potentiometer from the recorder inputs. Remove the batteries (two -D cells, 1.5 volt) from potentiometer before storage.

The recorder is now ready for use. Connect the (+) lead from the pyronometer to Hi terminal of the recorder and the (-) lead from the pyronometer to Lo terminal of the recorder and tie the Lo terminal to GND.

Notes

1. It is a good idea to clean the pyronometer every day. Abnormally low readings will result if the glass cover becomes dirty. It is also a good idea to check daily that the pyronometer is securely lashed, and that wires are safely out of the way from people working on deck.

2. Turn off the recorder unit at night to save paper.

3. It is a good policy to replace the mercury cell for the offset circuit in the Model 80 recorder each year (mercury cell #RM 625R 1.3V).

Introduction

Plants use light in the waveband 400-700 nm for photosynthesis. Within this waveband there is a direct relationship between the number of photons absorbed and photosynthetic activity regardless of the energy of the photon. Therefore, an accurate measurement of photosynthetically active radiation (PAR) requires the use of a quantum sensor which only responds to photons in the 400-700 nm waveband. The Licor model LI-192S underwater quantum sensor has an excellent quantum response for measuring PAR. It is used primarily for underwater light profiles. Air measurements are also taken for comparison with the Eppley pyronometer measurements of incident radiation (Section II.6.1) to allow an estimate of the daily PAR.

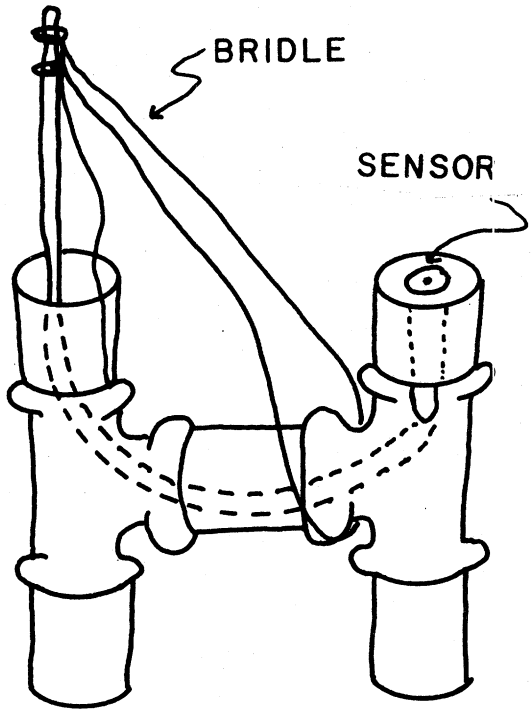

FIG. II.6.2.1 Underwater quantum sensor with pipe bracket mounting.

Equipment and Supplies

quantum sensor (LI=192S, LAMBDA Instrument Co., Lincoln Nebraska) encased in a pipe bracket to give it weight and assure an upright position underwater (Fig. II.6.2.1)
50 m underwater cable
LI-185 quantum meter with power supply
time constant circuit
Heathkit SR-204 strip chart recorder
Spare quantum sensor
batteries for LI-185 quantum meter (Eveready E135N, 6.75V)
chart paper
spare pens for Heathkit chart recorder

Procedure

The LI-185 quantum meter may be operated with the power supply or with batteries. When using the batteries turn the meter <u>off</u> between uses as the batteries will run down very quickly. The batteries should be checked before each use by selecting "batt." on the selector switch. A reading of 2.4 on the lower scale is the minimum acceptable value. To take a reading the meter is set on quantum and the range selector is adjusted to give a mid-scale reading (usually the 1000 or 3000 scale in air). Because of the glitter effect just below the surface of the water, records are made on the recorder (mark scale setting and depth on chart) and subsequently the average value for that depth is read from the chart. To reduce the noise due to glitter the time constant circuit, inserted between the meter and the recorder, is turned on. The

time constant circuit removes most of the glitter but requires several seconds to settle at each new depth.

Readings are made in the air just above the surface, at 1 m, 5 m, and reasonable intervals until the reading is less than 1% of the 1 m value. A minimum of five underwater measurements should be made. Depth is estimated from the 1 m interval markings on the cable. To account for the effect of immersing the quantum meter in water all underwater readings must be multiplied by 1.4.

Readings must be made during a consistent light regime. One half hr after sunrise, and 1/2 hr before sunset, the light is changing too rapidly to obtain accurate profiles. Special care must be taken on partially cloudy days.

The data are read from the strip chart recorder in $\mu Ein \cdot m^{-2} \cdot sec^{-1}$ (.3 on the 1000 scale = 300, etc.) and plotted on semi log paper as shown in Figure II.6.2.2.

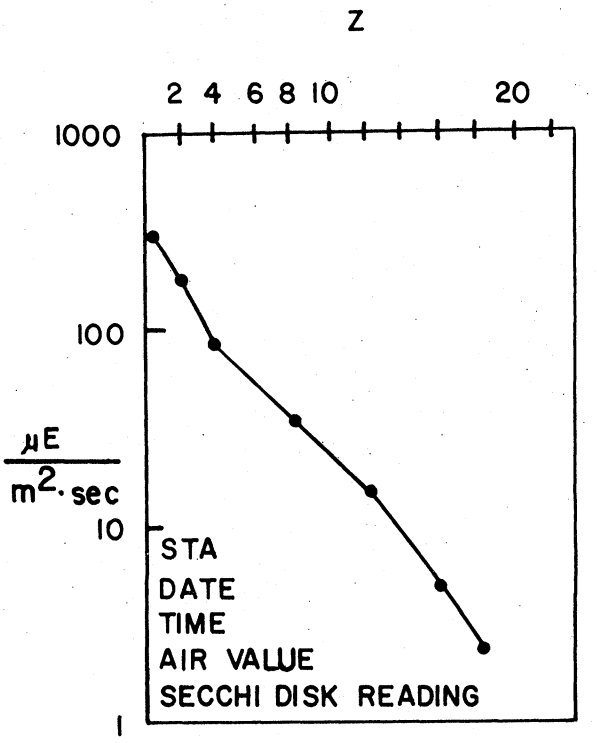

FIG. II.6.2.2 Example of a typical plot of PAR as a function of depth.

Notes

1. The quantum meter and Heathkit manuals are kept with the meter, and should be consulted if any questions arise.

2. The pipe fitting head and quantum sensor (Fig. II.6.2.1) should be checked before each use to insure that the sensor head is level and even with the pipe end that surrounds it, that the bridle is adjusted so that it carries all the weight of the pipe fittings, and that the sensor points straight up when suspended by the bridle. The white diffusion plate in the center of the sensor is very delicate, and should never be touched by a finger or hard object. It can be cleaned with a damp Kimwipe or lens paper, and should always be stored in such a way that there is no possibility of a hard object rubbing against it.

3. The quantum meter cable is a two-conductor underwater cable. It must be stored in figure 8's in its special box when not in use. Do not step on the cable or bend it sharply as this may break the wires inside. The cable is marked in meters, and the depth of the sensor is estimated by reading the mark at the surface of the water.

4. A program, LIGHT (B), written for an Alpha-16 minicomputer is available for detailed analysis of underwater light profiles.

Reference

Anon. 1974. Licor L1-1925 Underwater quantum sensor. Brochure C-973 Lambda Instruments Corp. Lincoln, Nebraska.

Introduction

The Secchi disk is a weighted white disk 30 cm in diameter attached to a line marked off in meters. The disk is lowered off the shady side of the ship and the depth that it disappears from view is recorded. There are a variety of different designs, 20 cm dia., black and white sections, etc., but the 30 cm dia. all white disk is the standard Secchi disk for Great Lakes limnology and for oceanography.

The objective in using the Secchi disk is to obtain a quick approximation of the extinction coefficient K_λ where K_λ is defined:

$$K_\lambda = 2.30(\text{Log } I_{(\lambda,z)} - \text{Log } I_{(\lambda,z+1)})$$

where $I_{(\lambda,z)}$ and $I_{(\lambda,z+1)}$ represent the radiation intensities of wave length λ on horizontal surfaces at the depths z and (z + 1) meters. Poole and Atkins (1929) have shown that in the English Channel the extinction coefficient for visible light can be approximated by:

$$K = 1.7/D$$

where D is the maximum depth of visibility in meters as determined by the Secchi disk. Beeton (1957) has shown that this relationship holds for the Laurentian Great Lakes.

Equipment

30 cm all white Secchi disk with 20 m hand line marked in meters

Procedure

The Secchi disk is lowered by hand on the shady side of the ship until the disk disappears from view. The depth at which the disk disappears is measured by the markings on the hand line, and recorded on the log sheet. On cloudy days when there are no visible shadows the disk may be lowered over either side of the ship. Secchi disk measurements can only be made during the daylight hours from 1/2 hr after sunrise to 1/2 hr before sunset.

References

Beeton, A. M. 1957. Relationship between Secchi disc readings and light penetration in Lake Huron. Trans. Amer. Fish. Soc. 87: 73-79.

Poole, H. H. and W. R. G. Atkins. 1929. Photoelectric measurements of submarine illumination throughout the year. J. Mar. Biol. Assoc., U.K. 16: 297-324.

III. LABORATORY PROCEDURES

Introduction

pH is defined as $-\log_{10}[H^+]$. In natural waters at a pH of 6.5 the most important buffer system controlling pH is the bicarbonate-carbonate system. The growth of phytoplankton is the most likely event to affect the balance of this system. As phytoplankton consume CO_2 in photosynthesis the balance in the bicarbonate-carbonate system shifts and the pH rises. Thus, pH is often used as an indicator of photosynthetic activity. Care must be taken in using this interpretation in developed areas as industrial wastes may also affect the pH.

Equipment and Supplies

pH meter (Corning model 110)
pH probes
buffers (pH 4.00, 7.00, and 10.00)
250 mL Nalgene beakers

Procedure

Each day, before the first sample is analyzed, the pH meter should be zeroed, calibrated, and sloped as described below. Sample water for pH measurement is obtained directly from the Niskin bottle with a 250 mL Nalgene beaker. The pH probes are immersed in the lake water in such a way that the level of the solution in the reference probe is above the level of the sample. The probes should never touch the bottom of the beaker. The solution is swirled around the probes by gently moving the beaker back and forth a few times. Then the operate button is pressed. The temperature switch should be in the ATC position. While the meter is equilibrating, the temperature of the sample is measured with a mercury thermometer marked in C°. The meter should then be switched to the expand mode to give a better indication of equilibration. This also allows the pH value to be accurately rounded off to two significant figures after the decimal point. Record the pH and laboratory temperature on the station log sheets.

Zero Adjustment

Each day, before the first station, the pH meter zero adjustment should be checked. Push the switch on the back of the meter to the calibrate position, put the meter in the operate-expand mode and adjust for a reading of 7.000 with the zero adjustment knob on the back of the meter.

Calibration

The pH meter should be calibrated each day prior to analyzing the day's first samples. The temperature knob should be set to the ATC position, and the electrodes immersed in a beaker of fresh pH 7.00 buffer. Put the meter in the operate mode, swirl the buffer solution around the electrodes by gently moving the beaker back and forth a few times, and take the temperature of the buffer solution. Turn the calibrate knob until the meter reads the correct value for the temperature of the buffer (see chart on back of buffer bottle). Switch the meter to expand scale and adjust further if necessary. Always give the pH meter enough time to equilibrate.

Slope Adjustment

The slope must also be adjusted daily. For reading pH, the

meter should be sloped up from 7.00 to 10.00. The procedures are the same as for calibration except that this time the slope knob is adjusted to obtain a correct reading for the pH 10.00 buffer.

Notes

1. The pH probes should be rinsed with buffer prior to calibrations, and after the calibration with the sample that is to be subsequently measured. The probes are never rinsed with distilled water between successive lake water samples. If rinsing is necessary always use a portion of the liquid that will be analyzed next.

2. The probes should be dipped into the sample (or buffer) in such a way that the solution in the reference probe is at a higher level than the solution being measured. Probes should never touch the bottom of the beaker.

3. The probes should always be left in lake water (never distilled water) with the pH meter in the standby mode when not in use.

4. A rapid fluctuation in the line voltage, similar to that which occurs when generators are switched or when changing from shipboard to shore power, causes the pH meter to overload (a blinking overload light is visible on the meter's face when in this condition). It is harmful for the pH meter to remain in this overload state for any period of time, so it should be checked periodically, especially in the evening after the ship has been docked. The meter is reset by pushing the operate button, and then the standby button.

5. Once the meter has equilibrated, it is a good idea to occasionally check the ATC by turning the temperature knob to the value obtained with the thermometer and comparing the resulting pH with the one obtained in the ATC mode.

Introduction

The bicarbonate-carbonate buffer system is the dominant buffer system in natural waters with a pH of approximately 6.5. The normal amounts of HCO_3^-, CO_3^{2-} and OH^- present in lake waters gives them a slightly alkaline pH (8.3). In exceptional cases unusually high concentrations of $H_3SiO_4^-$, $H_2BO_3^-$, NH_4^+, HS^- organic ions, and colloidal or suspended $CaCO_3$ may contribute significantly to the measured alkalinity. If there is no interference from these other substances then the alkalinity will be controlled solely by the bicarbonate-carbonate system and it is then possible to calculate the concentration of total CO_2 (free CO_2 + HCO_3^- + CO_3^{2-}) from the pH and alkalinity of the water (Golterman 1969). The calculation of total CO_2 is a necessary part of the ^{14}C-productivity method as described in III.4.2.

Equipment and Supplies

pH meter
pH probes
Acculute solution
buffers (pH 4.00 and 7.00)
2 oz. Nalgene bottles
125 mL Nalgene beakers
5 mL volumetric pipet
20 mL volumetric pipet

Procedure

A 20 mL volumetric pipet is rinsed with sample water then used to transfer 20.0 mL of sample water to a labeled 2-oz plastic alkalinity bottle. It is capped, shaken, and stored at room temperature until the end of the day when all alkalinity samples are analyzed together. These alkalinity bottles are prepared in advance by the addition of 5.0 mL of 0.010N HCl initially prepared from Acculute solution. The 0.010N HCl is added to each bottle with a Repipet.

Alkalinity values are measured with the pH meter. Prior to running the alkalinity samples the meter should be calibrated to pH 7.00 and sloped down to 4.00 (see III.1.1). To measure the alkalinity, the alkalinity bottle is shaken, its contents poured into a clean 125 mL Nalgene plastic beaker and the pH measured. If pH is less than 3.00 or between 3.00 and 4.00, record the value. If the pH is greater than 4.00 add more 0.010N HCl using a burette. Add 1.0 mL increments, mixing thoroughly, until the measured pH is between 3.00 and 4.00. Record the amount of acid added and the final pH value on the station log sheet.

Calculations

$$\text{Alkalinity} = \frac{Hi V_s + N V_a - Hf(V_s + V_a)}{V_s} \quad (1)$$

where

V_s = volume of sample taken
V_a = volume of acid taken (mL) of N normality
(Hi) = initial hydrogen ion concentration
(Hf) = hydrogen ion concentration after addition of acid
N = normality of acid

$Hi V_s$ is negligible for pH's greater than 6; therefore the form of the equation used is:

$$CO_3 \text{ alkalinity} = \frac{N V_a - Hf\,(V_s + V_a)}{V_s} \text{ meq/L} \quad (2)$$

Or in terms of C:

$$\text{alkalinity in mg C/L} = \text{meq/L} \times 12 \qquad (3)$$

Note

1. After the last alkalinity measurement has been run, the pH meter should be recalibrated at pH 7.00 and sloped to pH 10.00, preferably at least one hour before it is used for pH measurements. The meter should then be switched to the standby mode, with the probes left soaking in lake water. The pH probes should never be left in distilled water for any extended period of time.

Reference

Golterman, H. L. 1969. Methods for chemical analysis of fresh waters. IBP Handbook No. 8. Blackwell, Oxford. 166 pp.

Introduction

Specific conductance is a measure of the ability of a substance to conduct an electric current. The specific conductance of water is a function of the total number of ions present and the temperature of the water. Thus the measurement of specific conductance gives a summary value of the total number of ions present. However, it provides no indication as to the nature of the substances present.

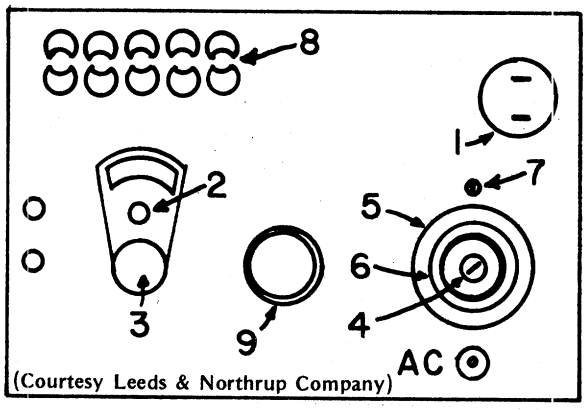

FIG. III.1.3.1 Leeds and Northrup conductivity bridge.

Equipment and Supplies

conductivity bridge (#4866 Leeds and Northrup Inc.)
conductivity probes
8-oz. wide mouth bottles
0.010N KCl standard solution

Procedure

Specific conductance is measured with the same water sample used for the measurement of pH. Immediately after recording the pH the water is used to rinse then fill a wide mouth 8-oz plastic bottle. These conductivity bottles are stored at room temperature until the end of the day when all conductivity measurements are made at one time.

The galvanometer of the conductivity bridge is zeroed with the power switch (Ac, Fig. III.1.3.1) in the off position and the galvanometer clamp (#2, Fig. III.1.3.1) in the unlock position. The adjustment knob (#3, Fig. III.1.3.1) at the base of the meter is turned until the meter reads "0," exhibiting no deflection.

The sample is shaken and its temperature is measured with a mercury thermometer. With the meter clamp locked, the conductivity electrode is placed gently into the beaker. Care should be taken to ensure that the electrode is completely immersed, and that no air bubbles remain inside the electrode to affect the measurement. The temperature dial (#5, Fig. III.1.3.1) is set to the sample water temperature and the galvanometer is released by pushing the clamp to the unlock position. This will cause the meter to deflect right or left. Knob number 9 (Fig. III.1.3.1) is turned until the galvanometer is nulled at center zero position. The specific conductance value is found by multiplying the number seen in the scale above knob 9, by the plugged valve on block 8. The galvanometer clamp is returned to the lock position and the procedure repeated with the next sample.

Standardization

A 0.010N potassium chloride solution is used for standardizing the conductivity cell. To prepare the standard, heat about 10 g of potassium chloride in a clean crucible until the salt begins to melt around the edges. Cool it in a desiccator. When cool, break up the crust which has formed with a

clean glass rod and weigh out 745.6 mg. Dissolve the weighed KCl in 1.000 L of freshly boiled distilled water. Do not allow the water or prepared solution to stand in an open vessel as gases from the air can change its characteristics. The specific conductance of the KCl solution can be found in tables in the conductivity bridge instruction manual, or the International Critical Tables.

Notes

1. Specific conductance measurements are preferably made after the ship has been tied up for the night. This procedure is preferred for two reasons: 1) ship motion causes the galvanometer needle to deflect, making accurate readings difficult, and 2) the temperature compensating circuit in the conductivity bridge is not perfectly linear over its entire range. Allowing all samples to warm up to near room temperature avoids this possible source of error.

2. When turning the power switch on or off the galvanometer clamp must always be in the lock position. Turning the conductivity bridge on and off causes a power surge which could burn out the galvanometer if it is not locked.

3. The conductivity electrode should be kept in water at <u>all times</u>. There is a plastic bottle filled with distilled water especially for this purpose. The top of the electode fits snuggly into the mouth of this bottle.

Reference

Anon. Directions std. 1306, issue 3 no. 4866 Conductivity Bridge, Leeds and Northrup, Philadelphia, PA.

Principle of the Method

This procedure for the determination of ammonia in lake water utilizes the automated colorimetric phenate method (U.S.E.P.A. 1976) based on the indophenol blue reaction. In the reaction, a basic medium facilitates conversion of ammonium ions to ammonia. The ammonia reacts with alkaline phenol to form a complex which is oxidized by hypochlorite, producing a blue color. Nitroferricyanide catalyzes the reaction and serves to prevent random variations in sensitivity caused by metal ions (Harwood and Huyser 1970). The initial introduction of the chelating agent EDTA serves to decomplex the ammonia and to complex the metals and prevent their precipitation and interference (Meyer 1957, Sharp and Mancy unpubl.).

Application and Range

This automated method is applicable to concentrations of ammonia in open lake waters in the range of 0.001 to 0.150 mg N/L when photometric measurement is made at 630 nm using a 50 mm tubular flow cell and a standard calibration of 5.00. Higher ammonia concentrations found in river water and near other point sources may require dilution.

Sample Preparation and Storage

Ammonia contamination can be a serious problem, especially with shipboard analysis where air temperature and circulation cannot be controlled. The sample containers should be tightly capped immediately after filtration with subsequent refrigeration below 5 ^{o}C. Analysis should be done within 24 hours and preferably as soon as possible after collection. Exposure of the sample to air during processing should be kept to a minimum.

Equipment and Supplies

The analysis is carried out on an Auto Analyzer II system as diagramed in Figure III.2.1.1. The normal sample rate is 30/hr.

Reagents

EDTA - Nitroprusside: Dissolve 1.86 g EDTA (ethylenedinitrilotetraacetic acid disodium salt) and 0.125 g of sodium nitroprusside (nitroferricyanide) in distilled-deionized water (DDW) and dilute to 500 mL. Adjust to pH 11 with NaOH (3 pellets/500 mL) and add 0.5 mL Brij-35 and refrigerate.

Alkaline Phenol: Dissolve 12.0 g NaOH in 400 mL DDW. Add 17.5 mL liquified phenol. Dilute to 500 mL with DDW and refrigerate.

Sodium Hypochlorite: Dilute 15 mL Chlorox (commercial bleach, 5.25% sodium hypochlorite) to 500 mL with DDW. Use bleach which is less than 3 months old. Prepare daily.

Standards

Stock Solution: Dissolve 0.3819 g of pre-dried (105^{o}C for 1 hour) NH_4Cl in DDW and dilute to 1 L (100 mg N/L). Prepare monthly and store in refrigerator. Preserve with 1 mL chloroform.

Intermediate Stock Solution: 10 ml stock solution diluted to 1000 ml DDW(1 mg N/L). Preserve with 1 mL chloroform and refrigerate.

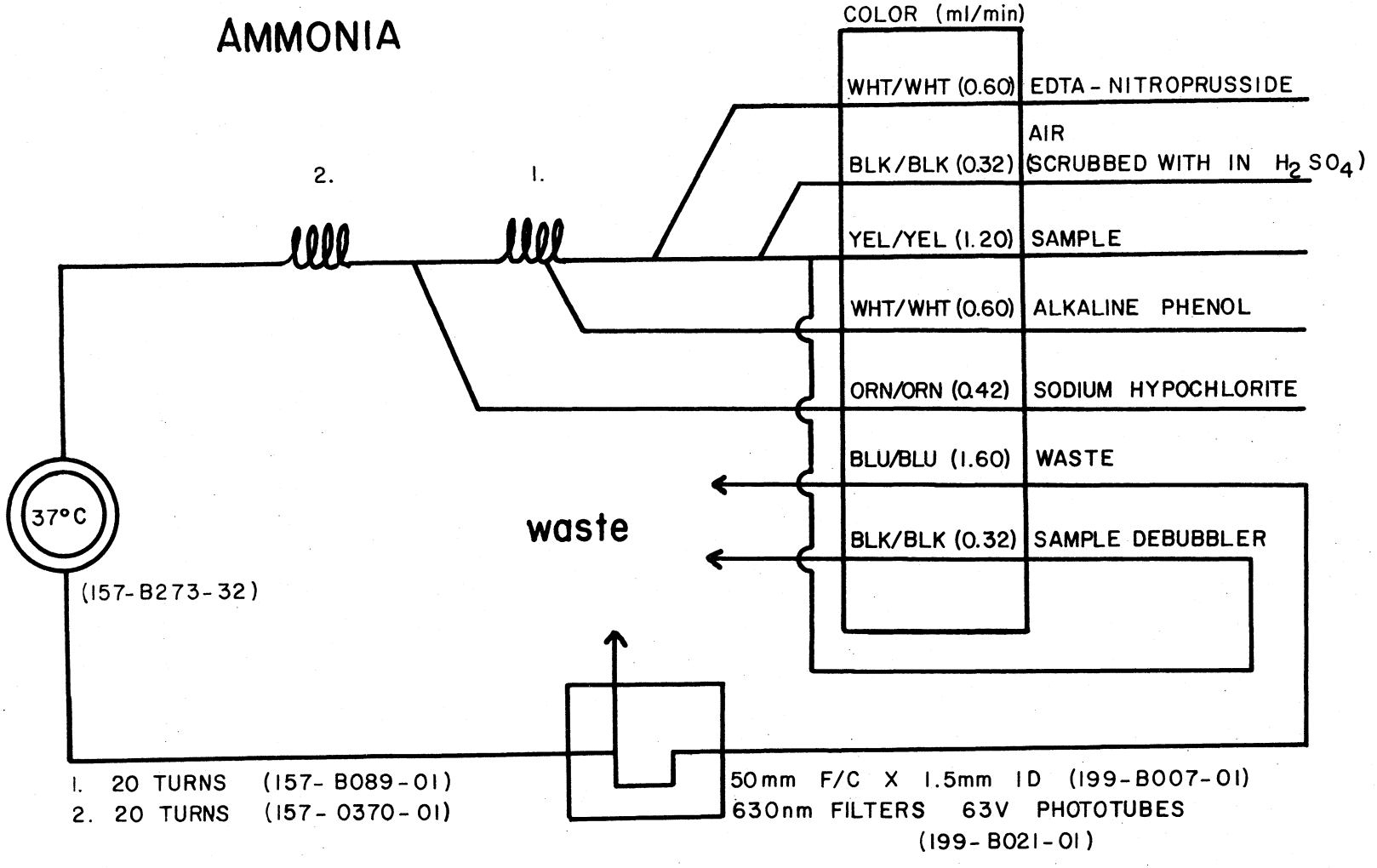

FIG. III.2.1.1 Ammonia manifold for AutoAnalyzer II system.

Working Standards for Lake Water (prepare daily):

Dilute the following volumes of intermediate stock solution to 100 mL with DDW:

mL Intermediate Stock/100 mL	NH_4-N, mg/L
1	0.01
2	0.02
3	0.03
4	0.04
5	0.05

Note

1. Despite the presence of EDTA, the Tygon tubing may become discolored with a coating of metal hydroxides. A long rinse with a strong complexing agent like oxalic acid will remove these deposits (Sharp and Mancy unpubl.).

References

Harwood, J. E. and D. J. Huyser. 1970. Automated analysis of ammonia in water. Water Research 4:695-704.

Meyer, H. 1957. The ninhydrin reaction and its analytical application. Biochemical Journal. 67:333.

Sharp, M. L. and K. H. Mancy. Unpublished. An introduction to the design operation and methodologies of an Auto Analyzer system. Univ. of Mich. School of Pub. Health, Ann Arbor.

U. S. Environmental Protection Agency. 1976. Manual of methods for chemical analysis of water and wastes. Office of Technology Transfer, Cincinnati, Ohio. pp. 168-174.

Principle of Method

This method is based on the initial reaction of chloride with mercuric thiocyanate to form mercuric chloride. The released thiocyanate (SCN^-) reacts with ferric ammonium sulfate to form a red complex, ferric thiocyanate, which is directly proportional to the original chloride concentration (O'Brien 1962; Zall et al. 1956). The procedure for this method is a variation of those used by the U.S. Environmental Protection Agency (1976) and the Inland Waters Directorate (1974).

Application and Range

For open lake water, measurements of chloride can be made in the range of 0.5 to 10.0 mg Cl/L. A 50 mm tubular flow cell is used with filters of 480 nm and a standard calibration of 2.50. River water may require dilution.

Sample Preparation and Storage

Chloride analysis requires no special handling or preparation of filtered lake water samples. If the analysis cannot be carried out immediately after collection then refrigeration is advisable.

Equipment and Supplies

The analysis is carried out on an Auto Analyzer II manifold as diagramed in Figure III.2.2.1. The normal sample rate is 30/hr.

Reagents

Mercuric Thiocyanate: Dissolve 0.7 g $Hg(SCN)_2$ in boiling distilled deionized water (DDW). Cool and dilute to 1 L with DDW. Filter three times through glass wool and store in polyethylene containers. The reagent is stable for one year.

Ferric Ammonium Sulfate (FAS): Add 77 mL concentrated HNO_3 to 500 mL DDW. Pulverize and dissolve 24 g $FeNH_4(SO_4)_2\ 12H_2O$ into this solution by stirring for at least 30 minutes. Filter through glass wool if necessary and store in a polyethylene container. The reagent is stable for one year.

Standards

Stock Solution: Dissolve 32.964 g of pre-dried (140°C for one hour) NaCl in DDW and dilute to 1 L (20,000 mg Cl/L).

Intermediate Stock Solution: 10 mL stock solution diluted to 1000 mL with DDW (200 mg Cl/L).

Working Standards for Lake Water: Dilute the following volumes of intermediate stock solution to 100 mL with DDW:

mL Intermediate Stock/100 mL	Cl mg/L
1	2.0
2	4.0
3	6.0
4	8.0
5	10.0

References

Inland Waters Directorate. 1974. Analytical Methods Manual. Water Quality Branch, Inland Waters Directorate, Environment Canada, Ottawa.

O'Brien, J. E. 1962. Automatic analysis of chlorides in Sewage. Waste Engineering 33:670-672.

U.S. Environmental Protection Agency. 1976. Manual of methods

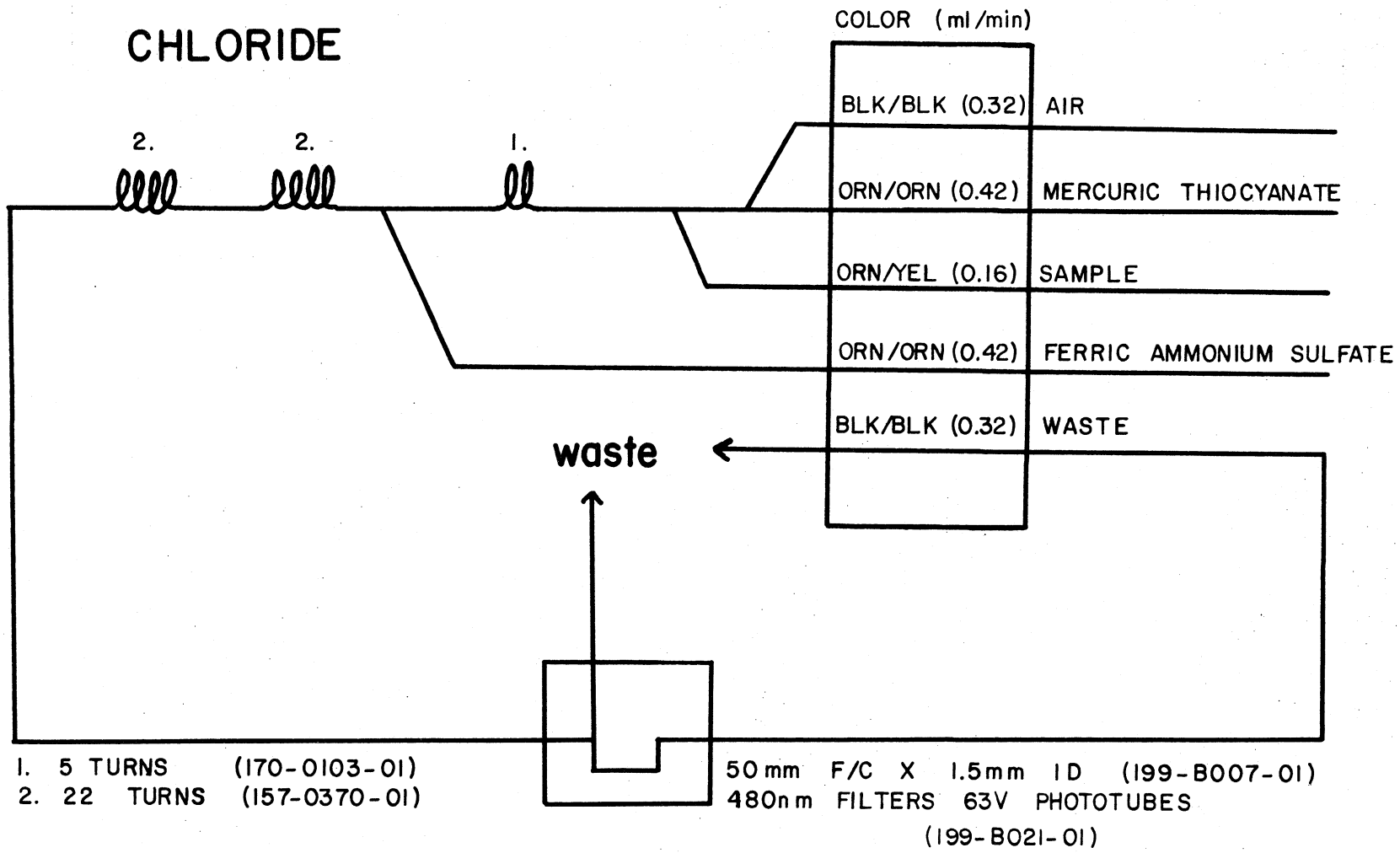

FIG. III.2.2.1 Chloride manifold for AutoAnalyzer II system.

for chemical analysis of water and wastes. Office of Technology Transfer, Cincinnati, Ohio. pp. 31-34.

Zall, D. M., D. Fisher and M. Q. Garner. 1956. Photometric determination of chlorides in water. Anal. Chem. 28: 1665.

Principle of the Method

This method for the determination of nitrate and nitrite utilizes the reaction whereby nitrate is reduced to nitrite by a copper-cadmium reductor column. The nitrite ion then reacts with sulfanilamide under acidic conditions to form a diazo compound. This compound then couples with N-1-napthylethylenediamine dihydrochloride to form a reddish-purple azo dye (Wood et al. 1967; Armstrong et al. 1967).

When present in high concentration, metal ions may produce a positive error, i.e., divalent mercury and divalent copper may form colored complexes having absorption bands in the region of color measurement for nitrate.

Application and Range

The range for this method in open lake waters is 0.01 to 1.0 mg N/L which is the normal range encountered in lake water. A 50 mm flow cell is used with interference filters of 550 nm and a standard calibration of 1.00.

Sample Preparation and Storage

Samples should be filtered and analyzed as soon as possible. If this cannot be done immediately, they should be refrigerated at <5 ^{o}C or preserved with 1 drop of chloroform per 100 mL of sample.

Equipment and Supplies

The analysis is carried out on an Auto Analyzer II manifold as diagramed in Figure III.2.3.1. The normal sample rate is 30/hr.

Reagents

Ammonium Chloride: Dissolve 10 g NH_4Cl in distilled deionized water (DDW) and dilute to 1 L. Add 0.5 mL Brij-35.

Color Reagent: To 1500 mL DDW add 200 mL conc. H_3PO_4 and 20 g sulfanilamide. Dissolve completely. Add 1 g N-1-napthylethylenediamine dihydrochloride and dissolve. Dilute to 2 L with DDW, store in an amber reagent bottle and refrigerate. If the color reagent develops any color (pink) discard and prepare fresh reagent.

Preparation of Reductor Columns: Wash several grams of course cadmium filings with 10% HCl in a beaker and rinse well with DDW. Decant rinse water and pour in 2% $CuSO_4$ solution. Swirl and let stand for 15-20 minutes, then rinse well with DDW (at least 12 rinses). Decant rinse water and replace with NH_4Cl (10g/L DDW) and pack the glass tubing (90 mm X 5 mm I.D.) with the cadmium in this solution. Pack ends of tubing with glass wool and keep prepared columns submerged in NH_4Cl solution until ready for use. Avoid exposure of the cadmium to air during preparation of the column and its placement on line.

Standards

Stock Solution: Dissolve 7.218 g of pre-dried (105^{o}C for one hour) KNO_3 into 1 L DDW (1000 mg N/L). Add 1 mL of chloroform as a preservative and refrigerate.

Intermediate Stock Solution: Dilute 10 mL of stock solution to 1000 mL DDW (10 mg N/L). Preserve with 1 mL chloroform and refrigerate.

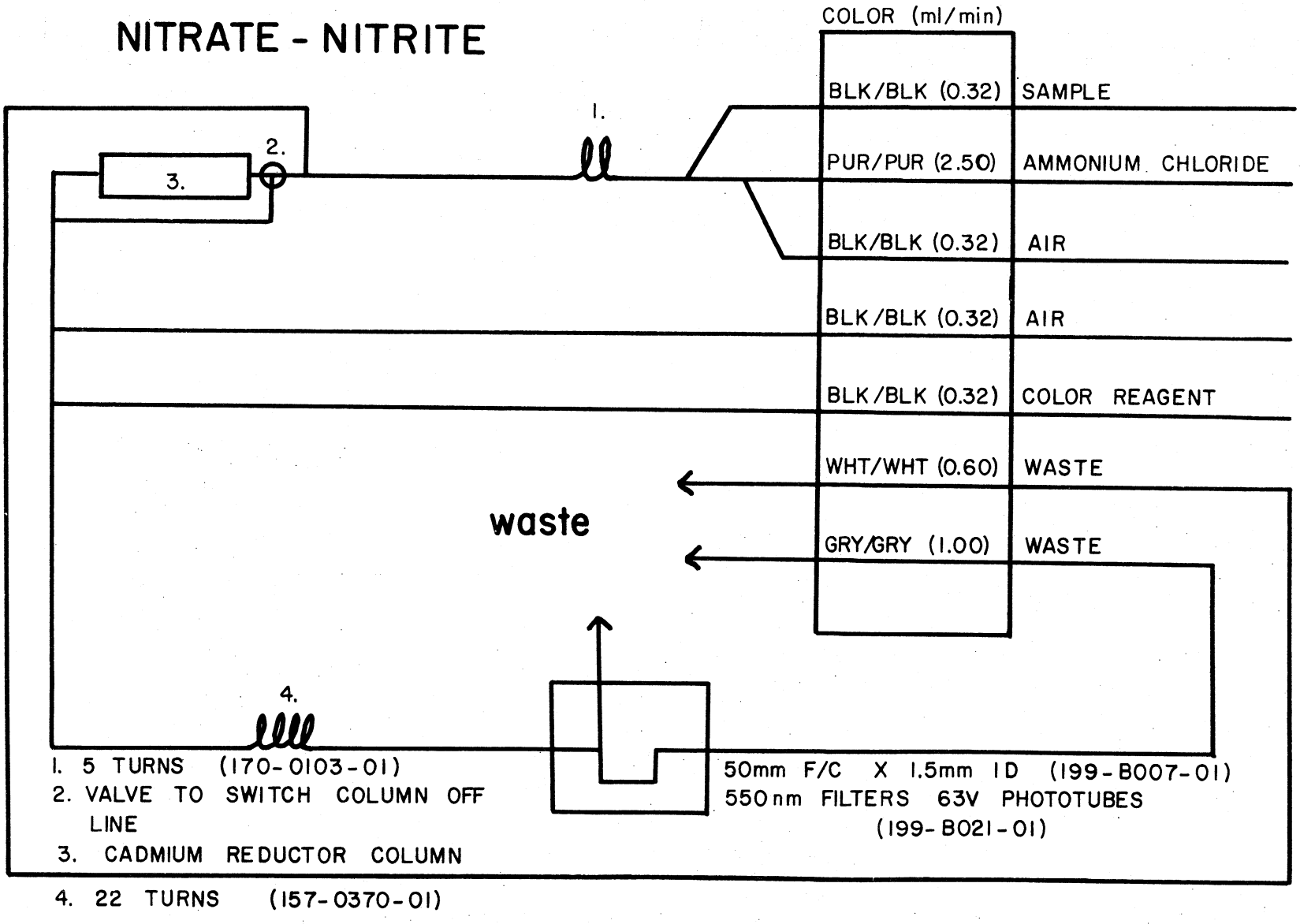

FIG. III.2.3.1 Nitrate-nitrite manifold for AutoAnalyzer II system.

Working Standards for Lake Water: Dilute the following volumes of intermediate stock solution to 100 mL with DDW:

mL Intermediate Stock/100 mL	NO_3-N mg/L
1	0.1
2	0.2
3	0.3
4	0.4
5	0.5

Notes

1. In order to determine nitrate levels, nitrite must be subtracted from the total (nitrate and nitrite) determined by this analysis. The nitrite value can be determined by eliminating the reductor column from the manifold. This is not done routinely, as the concentrations of nitrite in open waters of the Great Lakes are generally quite low.

2. The reductor column must be clean and have good flow characteristics for the system to operate satisfactorily. Colloidal copper and air are the primary contaminants to avoid. Turn the column off after the last sample before starting the water rinse. Ten percent HCl will destroy the column, therefore be certain the column is off before rinsing the system with 10% HCl. For initial activation of the reductor column, a high standard should be pumped through the system for about 1/2 hour.

3. The efficiency of the reductor column has been found to be 99%. It should be checked periodically by switching the column off and analyzing nitrite standards. 100 mg/L NO_2 stock is made by dissolving .4926 g $NaNO_2$ in 1 L DDW. Dilute 5 mL of 100 mg/L stock to 1 L (0.5 mg/L). A 0.5 mg/L NO_2 sample analyzed with the column off should yield the same results as 0.5 mg/L NO_3 sample with the column on.

References

Armstrong, F. A. J., C. R. Stearns and J. D. H. Strickland. 1967. The measurement of upwelling and subsequent biological processes by means of the Technicon AutoAnalyzer and associated equipment. Deep-Sea Res. 14: 381-389.

Wood, E. D., F. A. J. Armstrong and F. A. Richards. 1967. Determination of nitrate in sea water by cadmium-copper reduction to nitrite. J. Mar. Biol. Assoc. U. K. 47: 23-31.

Principle of Method

Orthophosphate reacts with ammonium molybdate and antimony potassium tartrate in an acid medium to form an antimony-phosphomolybdate complex, which is reduced by ascorbic acid to a phosphomolybdate blue complex. This complex is read at 880 nm by a colorimeter equipped with red sensitive phototubes (Murphy and Riley 1962; Armstrong et al. 1967).

Application and Range

The range for this method is 1 to 100 µg PO_4-P/L. Photometric measurement is made at 880 nm using a 50 mm flow cell and a standard calibration of 6.00.

This procedure is designed for low concentrations of orthophosphate present in the open waters of the Great Lakes. Higher concentrations which may be found in and around rivers may be analyzed by selecting a lower standard calibration.

Sample Preparation and Storage

The analysis is run on filtered samples. The filtrate should be kept refrigerated at 4°C until analysis is commenced. If samples are not analyzed within eight hours of collection, they should be preserved by freezing. Just before analysis, frozen samples should be thawed at 15-30°C in a hot water bath. Heat the samples just long enough to thaw and keep them chilled until analyzed.

Equipment and Supplies

The analysis is carried out on an AutoAnalyzer II System as diagramed in Figure III.2.4.1. The normal sample rate is 30/hr.

Reagents

Ascorbic Acid Reagent: In a 100 mL volumetric flask, dissolve 2.0 g of U.S.P. quality ascorbic acid in approximately 75 mL distilled-deionized water (DDW). Add 5 mL acetone and dilute to 100 mL with DDW. Add 3 drops Levor IV. The solution is reported to be stable for one week at 4°C (Amer. Pub. Health Assoc. 1976). However, for best results, prepare the reagent fresh daily.

Sulfuric Acid Solution (4.9 N): In a 2000 mL volumetric flask, slowly pour, in increments, 274 mL conc. analytical reagent grade sulfuric acid (H_2SO_4) into 1500 mL DDW. When the solution is cool dilute it to 2000 mL with DDW. Store in a 2-L bottle. The solution is stable indefinitely.

Antimony Potassium Tartrate Solution: In a 1000 mL volumetric flask dissolve 3.0 g analytical reagent antimony potassium tartrate ($K(SbO)C_4H_4O_6$ 1/2 H_2O) in 900 mL DDW. Mix and dilute to 1 L with DDW. Store in a dark glass bottle in the refrigerator. The solution is stable for many months (Amer. Pub. Health Assoc. 1976).

Ammonium Molybdate Solution: In a 1000 mL volumetric flask dissolve 40.0 g analytical reagent grade ammonium molybdate ($(NH_4)_6Mo_7O_{24}$ 4 H_2O) in 900 mL DDW. Mix and dilute to 1 L with DDW. Store in a dark plastic bottle in the refrigerator. This solution is stable until a precipitate forms.

Ammonium Molybdate Working Reagent: Combine the following reagents in order, mixing after the addition of each reagent.

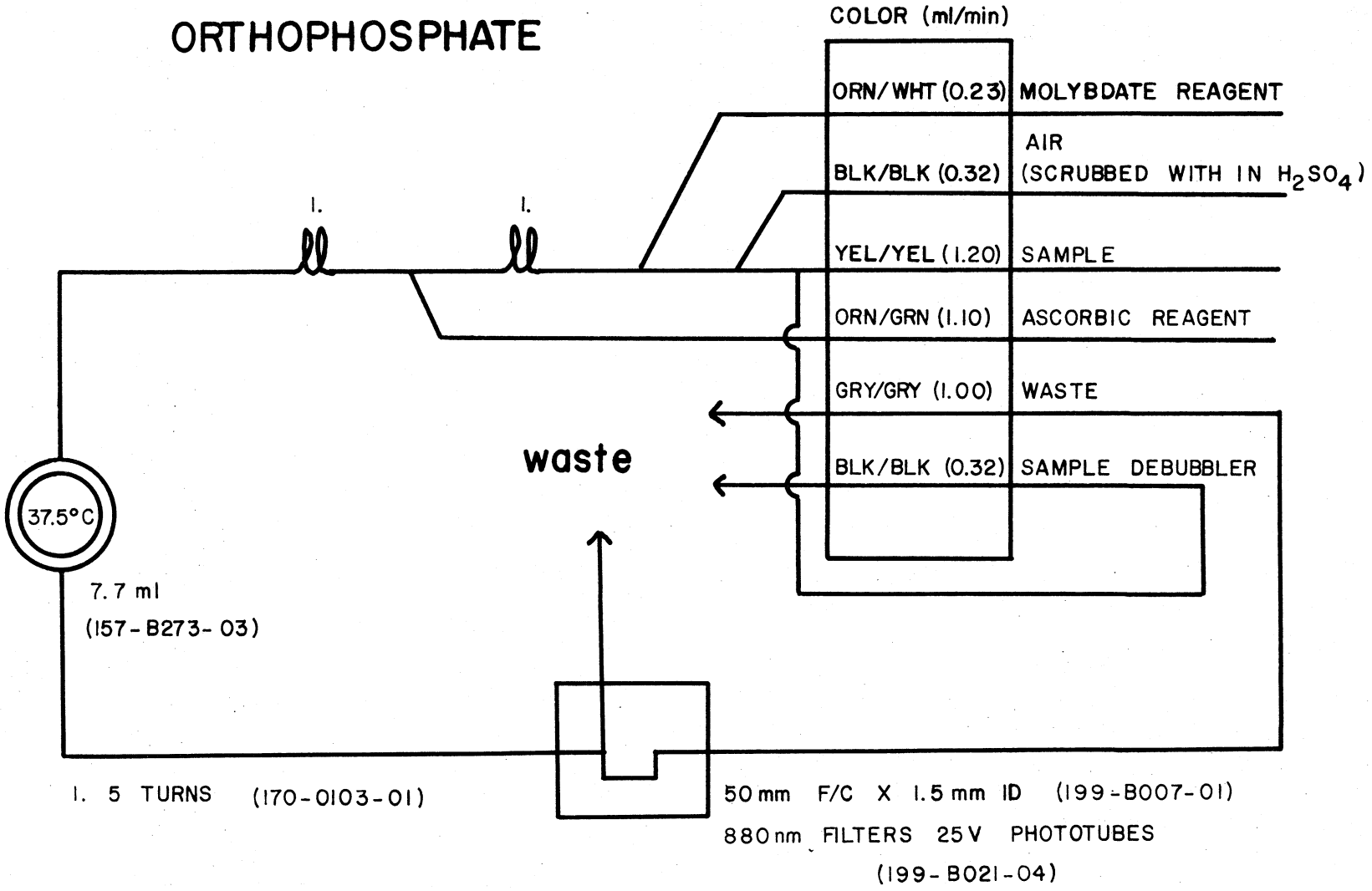

FIG. III.2.4.1 Orthophosphate manifold for AutoAnalyzer II system.

Combined Reagent	
Sulfuric acid solution, 4.9 N	150 mL
Ammonium molybdate solution	45 mL
Antimony potassium tatrate solution	15 mL

Any turbidity should be eliminated by continued mixing. Prepare daily, preferably with reagents that have reached room temperature.

Standards

Stock Solution: Dissolve 0.8787 g of pre-dried (105°C for one hour) primary standard potassium dibasic phosphate (KH_2PO_4) in 1000.0 mL DDW. Store in a dark bottle with 1 mL chloroform and refrigerate. The solution is stable for many months (Strickland and Parsons 1972).

Intermediate Stock Solution: Dilute 10.0 mL of stock solution to 1000.0 mL with DDW (2 mg PO_4-P/L). Store in a dark bottle with 1 mL chloroform and refrigerate. The solution may be stable for many weeks but, for greatest accuracy it should be made fresh about once every 10 days (Strickland and Parsons 1972).

Daily Stock Solution: Dilute 10.0 mL of stock solution to 100.0 mL with DDW (0.2 mg PO_4-P/L). Prepare daily.

Working Standards: Dilute the daily stock solution with DDW in 100 mL volumetric flasks. To prepare the working standards:

mL Daily Stock solution/100 mL	µg PO_4-P/L
1.0	2.0
2.0	4.0
3.0	6.0
4.0	8.0
5.0	10.0

Notes

1. Phosphate contamination from sample handling can be reduced by washing sample bottles and all glassware with 10% v/v HCl and rinsing thoroughly with DDW. Do not use commercial detergents.

2. Phosphate adsorbs to its container, especially polyethylene, hence samples should be analyzed as soon as possible.

3. The water bath temperature should be kept at 37°C for best sensitivity.

4. The phosphomolybdate complex will coat the AutoAnalyzer system, especially any plastic tubing. Reaction temperatures higher than 37°C will increase the tendency of the complex to coat the system. Coating should be kept to a minimum by using only glass tubing, by keeping the reaction temperature at 37°C and by adding Levor IV to the ascorbic acid reagent. Otherwise there will be increased carryover from one sample to another, especially at high concentrations.

References

American Public Health Assoc. 1976. Standard Methods for the Examination of Water and Waste Water. 14th edition.

Armstrong, F. A. J., C. R. Stearns and J. D. H. Strickland. 1967. The measurement of upwelling and subsequent biological processes by means of the Technicon AutoAnalyzer and associated equipment. Deep-Sea Res. 14: 381-389.

Inland Waters Directorate. 1974. Analytical methods manual. Water Quality Branch, Inland Waters Directorate, Environment Canada, Ottawa.

Murphy, J. and J. P. Riley. 1962. A modified single solution method for the determination of phosphate in natural waters. Anal. Chim. Acta. 27: 31-36.

U.S. Environmental Protection Agency 1974. Manual of methods for chemical analysis of water and wastes.

Strickland, J. D. H. and T. R. Parsons. 1972. A practical handbook of sea water analysis J. Fish. Res. Board Can. 167. 311 pp.

Principle of the Method

This automated procedure for the determination of soluble silicates is based on the formation of a greenish-yellow silicomolybdate color complex. Ammonium molybdate in acidic solution reacts with dissolved silica and any phosphate present to produce heteropoly acids. The introduction of oxalic acid breaks down the molybdophosphoric acid but not the molybdosilicic acid. Ascorbic acid is then added to reduce the silicomolybdate complex to a stable heteropoly blue complex. The blue color is more intense than the yellow and is proportional to the original concentration of "molybdate-reactive" silica (Amer. Pub. Health Assoc. 1976).

Interference from phosphates is eliminated by oxalic acid. However, large amounts of iron, tannin, color, turbidity, or sulfide may also cause interference.

Application and Range

The working range of this method is 0.05 to 2.0 mg SiO_2/L with interference filters of 660 nm. A 50 mm tubular flow cell is used in this analysis and a standard calibration of 2.50.

Sample Handling and Storage

Samples should be filtered immediately upon collection and are stored in polyethylene containers. Low concentrations of silica as found in open waters of the Great Lakes require that analysis be done within eight hours of collection. If this is not possible freeze the samples. Then samples which have been frozen should stand at least 12 hours at room temperature before beginning analysis.

Equipment and Supplies

The analysis is carried out on an AutoAnalyzer II manifold as diagramed in Figure III.2.5.1. The normal sample rate is 30/hr.

Reagents

Ammonium Molybdate Reagent: Dissolve 10 g $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$ in 1 L of 0.1 N H_2SO_4 (2.8 mL concentrated sulfuric acid/L, sp. gr. 1.84). Refrigerate.

Oxalic Acid: Dissolve 50 g $H_2C_2O_4 \cdot 2H_2O$ in 900 mL distilled-deionized water (DDW) and dilute to 1 L.

Ascorbic Acid Reagent: Dissolve 4.4 g ascorbic acid, U.S.P. ($C_6H_8O_6$) in 200 mL DDW containing 12.5 mL acetone. Dilute to 250 mL with DDW, add three drops of Levor IV and refrigerate.

Standards

Stock Solution: Dissolve 9.460 g $Na_2SiO_3 \cdot 9H_2O$, reagent grade sodium metasilicate, in DDW and dilute to 1 L (2000 mg SiO_2/L). Store in polyethylene bottles and refrigerate.

Intermediate Stock Solution: Dilute 10 mL stock solution to 1000 mL with DDW (20 mg SiO_2/L). Store in polyethylene bottles and refrigerate.

Working Standards for Lake Water: Dilute the following volumes of intermediate stock solution to 100 mL with DDW:

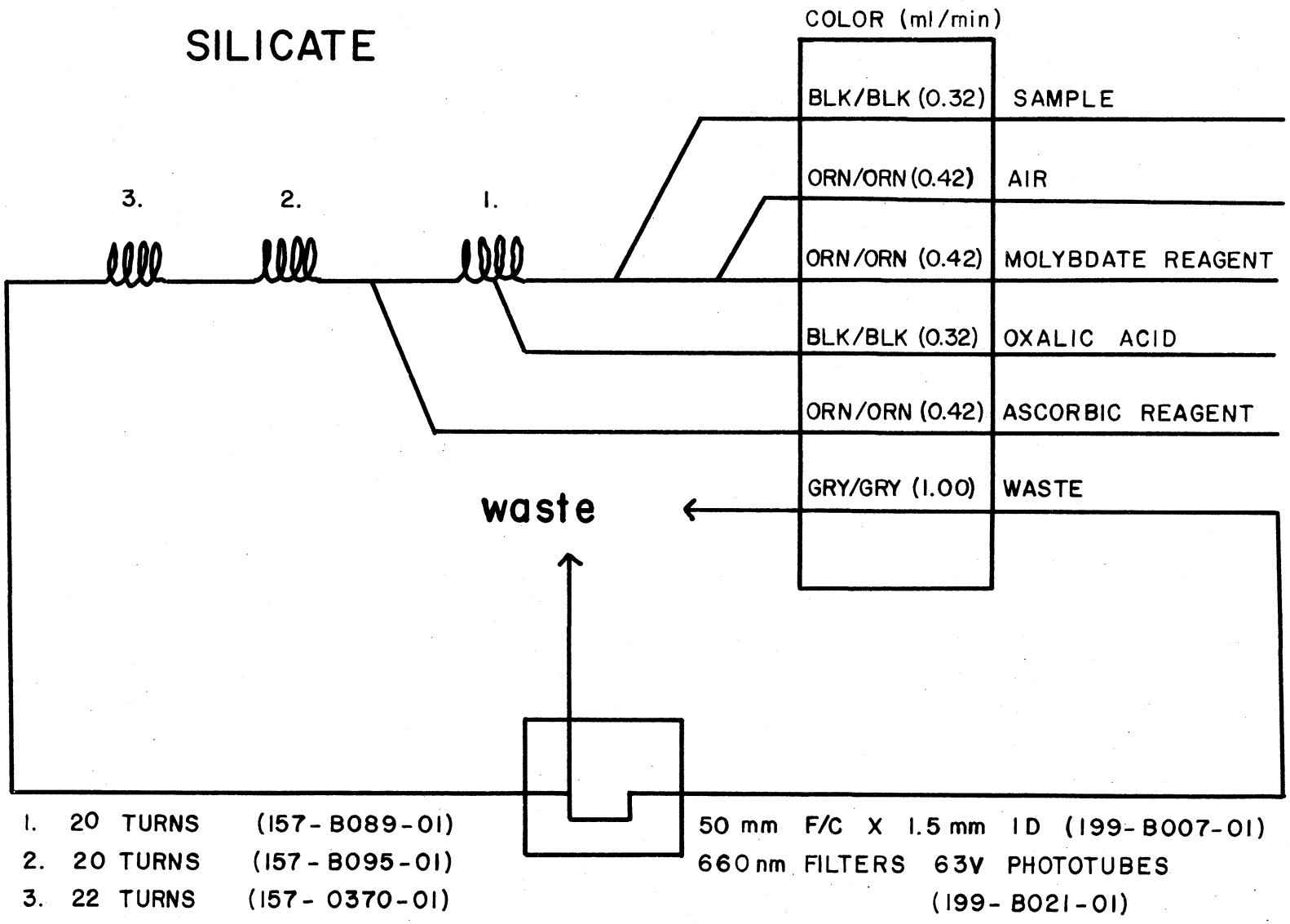

FIG. III.2.5.1 Silicate manifold for AutoAnalyzer II system.

mL Intermediate Stock/100 mL	SiO_2 mg/L
1	0.2
2	0.4
3	0.6
4	0.8
5	1.0

Notes

1. The ammonium molybdate reagent should be checked periodically for the development of a white precipitate and should be replaced with a fresh solution if a precipitate is present. Also, the ammonium molybdate reagent line should be rinsed every third day with 20% KOH to remove any deposit in the tubing and flow cell.

Special care must be taken to ensure that this rinse does not mix with the 10% HCl rinse solution used to clean the sample lines as a violent reaction could result.

2. All reagents samples and standards should be stored in polyethylene containers.

Reference

American Public Health Assoc. 1976. Standard Methods for the examination of water and waste water. 14th edition.

Introduction

This section describes a five channel Technicon AutoAnalyzer II system used in the analysis of ammonia, chloride, nitrite and nitrate, orthophosphate, and silicate. Descriptions of the analyses may be found in Sections II.2.1-5 and are not included in this section.

The components of this system have been organized to minimize transportation problems and facilitate set-up for shipboard analysis. The proportioning pumps and manifolds are assembled in a large plywood box, with the colorimeters arranged in a second box. The boxes are shallow and the bottoms of the boxes have a 1" flange on the front for clamping to a lab bench on shipboard. The system is fully assembled and tested in the laboratory. Then the sections are disconnected, transported to the ship, and reconnected. The system can be secured on shipboard, connected, and made operational in a few hours.

This system has been specifically assembled and calibrated to analyze open water samples from the Laurentian Great Lakes. Water samples taken from rivers, small lakes, and waste water may differ significantly in nutrient concentrations and require dilution or recalibration of the optical settings.

The description that follows is designed to supplement the information provided in Sections II.2.1-5 and in the Technicon manuals for the different pieces of equipment. The particular emphasis of this section is on packing and preparation for cruises and on recognizing and avoiding and/or correcting the most common problems encountered in operating an AutoAnalyzer system.

Description of Major Components

Sampler: The sampler used is a standard Technicon AutoAnalyzer Sampler II with a modified sampling arm and turntable to allow the use of 1-oz Nalgene widemouth linear polyethylene bottles to hold the samples instead of sample cups (Lee Conway pers. comm.). Samples are filtered and then placed directly in the 1-oz sample bottles, thus avoiding an additional transfer of the sample and the possibility of contamination from the sample cups. The sample probe samples from near the bottom of the bottle and the top half of the sample is not used. This greatly decreases the chance for contamination from the air which is a problem with the Technicon sample cups where most of the sample is used.

The automatic shutoff feature of the Sampler II has been removed. Therefore, it is advisable to place 2 or 3, 1-oz bottles with DDW at the end of the sample run to give some allowance for shutting off the sampler after the last sample but before sampling air.

AA II Proportioning Pump: The proportioning pump meters the flow of sample, reagents, and air through the AutoAnalyzer system. Use of the air bar is not necessary since bubble size and frequency can be controlled with the flow calibrated pump tubes. Keep pump transmission tube joints tight; faulty connections may allow water to leak into the bottom of the pump which sets off an alarm. If the alarm rings, turn off the pump and clean with Kimwipes and a hemostat. Do not take the pressure plate off the

pump tubes unless the reagents have been taken off line to prevent reverse flow and contamination of the reagents. Always switch the NO_3 column off line before removing the pressure plate or rinsing with HCl or distilled-deionized water.

Change all pump tubes before each cruise and/or after about 20-30 days use. For optimum performance of the pumps observe the preventive maintenance schedule which can be found in the proportioning pump instruction manual.

Manifolds: Plumbing diagram for each analysis is given in the section on that analysis. Pathways should follow the shortest, most unobstructed route possible, but there should not be any connections under tension or pressure as these will eventually come apart.

Colorimeter: Standard AutoAnalyzer II colorimeters (Fig. III.2.6.1) are used to assess the color intensity of the final solutions. The proper filters, flow cells, and standard calibration settings are given in the instructions for each individual method. Aperture control "A" controls the sample aperture, control "B" controls the blank aperture. Clockwise rotation opens the aperture. For maximum performance "A" should be open as far as possible and the baseline adjusted with "B" and the baseline adjust.

Recorder: Maintenance of recorders includes replacing fuses and replenishing chart paper and ink. After changing chart paper check the zero and full scale adjustments. If the pen is not empty but no ink flows, prime the pen by applying a vacuum to the pen tip with a syringe and transmission tubing. For more serious problems consult the owner's manual for the recorder.

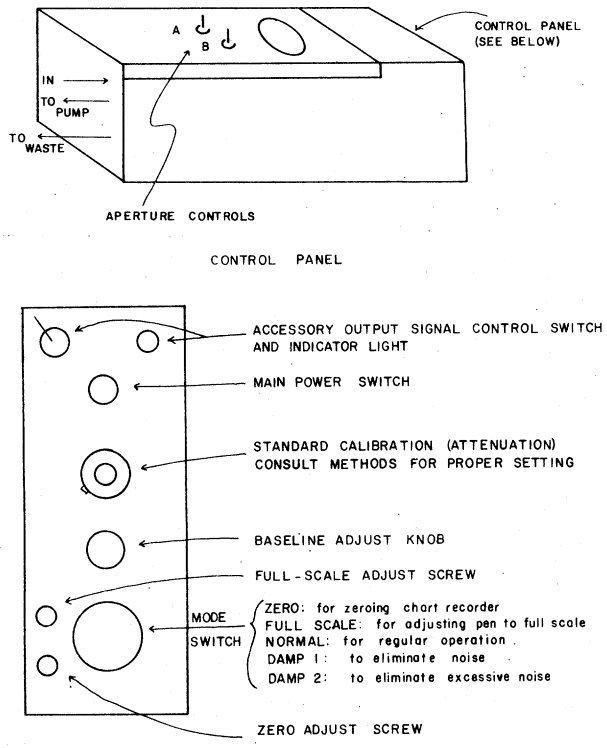

FIG. III.2.6.1 AutoAnalyzer II colorimeter.

Start-up Procedure

Turn on the colorimeters, heating baths, and electronic circuits of recorders at least 1 hour before use. Turn on the pumps and run 10% HCl through the sample lines for 5-10 minutes to wash the plumbing system (NO_3 Cd-column must be off line). Put the sample probe back into rinse water and flush the system for 5 minutes. Approximately every third day run 20% KOH through the SiO_2- molybdate reagent line to remove deposits in tubing. Never rinse with KOH and HCl at the same time; a violent reaction will result.

After the HCl rinse has been flushed out of the system, put the

reagents on line. All reagent containers must be covered at all times to prevent contamination. Check if there is enough reagent to do a day's work and prepare additional reagents as needed. After five minutes, if certain that no more bubbles or HCl rinse can get to NO_3 column, turn on the column at the bypass manifold. Clear the flow cells of bubbles by pinching and releasing the tubing connected to outflow end of the flow cells. Turn on recorder chart drives and allow the system to stabilize. When a straight baseline is obtained for at least five minutes the system is ready to run standards and samples.

Place standards, two water blanks, and samples on the carousel. Use the 20 or 30 - sample-per-hour-cam 2/1: sample/rinse ratio. The faster sample rate is preferred if sufficiently complete peaks are obtained at that sample rate.

Shut-down Procedure

When a baseline has been established after the last sample, put the reagent transmission line in distilled deionized water (DDW) and rinse the system for ten minutes. This DDW rinse bottle must be different from the sampler rinse to avoid contaminating the sampler rinse. After the system has been flushed with DDW, turn off the recorder chart drives and the proportioning pumps. Refrigerate those reagents that require refrigeration and all standard solutions. If the system is not to be used for several days all electrical circuits should be turned off.

Mixed Standards

This section describes the procedure for the preparation of combined stock and working standard solutions. A detailed discussion of the preparation and storage of individual standard solutions can be found in the appropriate section for each chemical analysis. All glassware used in the preparation of standards are class A volumetrics which have been rinsed with 10% HCl and then DDW. All weight measurements are based on an assay of 100.0% and must be corrected if the assay of a particular chemical is below this percentage. All stock chemicals except sodium metasilicate are oven dried and cooled in a desiccator before weighing. The silica standard is kept in a desiccator after opening.

Combine 7.218 g KNO_3, 9.460 g $Na_2SiO_3 \cdot 9\ H_2O$, and 32.964 g NaCl in 1 L DDW. Dilute 10 mL of that solution to 1000 mL with DDW for the Intermediate Stock Standard. Preserve with 1 mL chloroform.

Dissolve 0.8787 g KH_2PO_4 in DDW and dilute to 1 L. Dilute 10 mL to 1000 mL with DDW for the Primary Intermediate and then 10 mL of the Primary Intermediate to 100 mL for the Secondary Intermediate Stock Standard. Preserve with 1 mL chloroform.

Dilute 1, 2, 3, 4, 5 mL of NO_3-SiO_3-Cl Intermediate stock with approximately 50 mL DDW in each of 5 100-mL volumetric flasks. Next add 1, 2, 3, 4, and 5 mL of the PO_4 Secondary Intermediate Stock to the same flasks. Dilute to 100 mL with DDW. This will yield working standards of the following concentrations in mg/L:

NO_3	0.10	0.20	0.30	0.40	0.50
SiO_2	0.20	0.40	0.60	0.80	1.00
Cl	2.00	4.00	6.00	8.00	10.00
PO_4	0.002	0.004	0.006	0.008	0.010

A separate ammonia standard is made because the combined standard has a measurable amount of ammonia contamination. Dissolve 0.3819 g NH_4Cl in 1 L DDW. Dilute 10 mL to 1000 mL with DDW for the intermediate stock solution. Preserve with 1 mL chloroform. To prepare working standards, dilute the intermediate stock: 1, 2, 3, 4, and 5 mL each to 100 mL with DDW. Final concentrations in mg/L:

NH_3	0.01	0.02	0.03	0.04	0.05

Refrigerate all intermediate and working standards. Working standards are stable for one day.

For each set of standards run a concentration vs. absorbance curve, which should be plotted, labelled, and dated, and kept in a standards notebook. A "Std/Eval" program on the Alpha-16 minicomputer is also available. It tests the linearity of the standard curve. A maximum of 5 percent deviation is acceptable. Record the values of the slope and y-intercept.

Inline Filtering System and Shipboard Underway Analysis.

Normally discrete nutrient samples are filtered through a presoaked Millipore filter before analysis to remove particulate material. For continuous mode operation for making vertical pump profiles or underway maps, it is necessary to use an inline filtering system instead (Fig. III.2.6.2).

Soak the filters and rinse the filter holder in DDW. Clamp the filter in the center of the holder. Connect plumbing as diagramed below (Fig. III.2.6.2). Lake water is pumped from the debubbler with two purple pump tubes. Air is added with a black pump tube. The

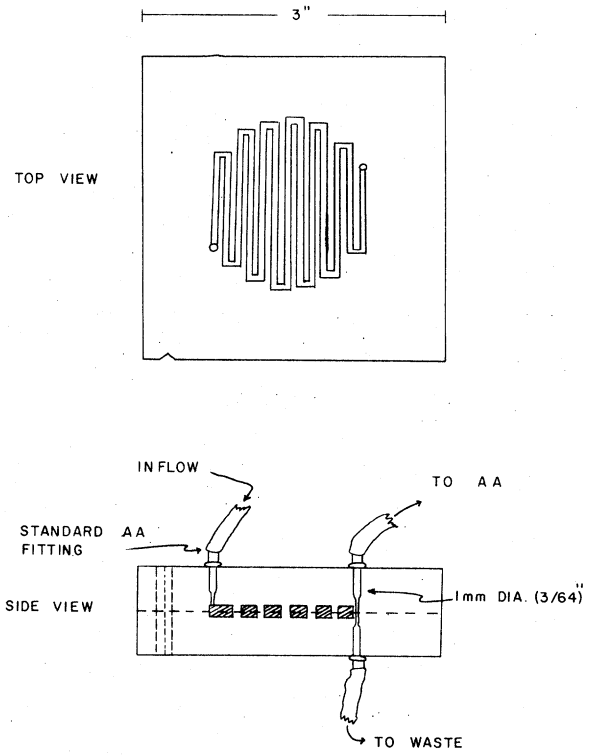

FIG. III.2.6.2 AutoAnalyzer inline filter unit.

sample outflow from the filter should be connected directly to the five channel sample splitter.

Run DDW through the filter inflow line until a steady baseline is obtained. This baseline should be the same as the baseline obtained without the filter inline. Then run a standard curve by placing the inflow line in each of three standards for four minutes each. Rinse with DDW for at least four minutes, then connect inflow line to lake water.

Be certain that the two inside surfaces of the filter holder are clean, that the filter is in the center, and that the clamps are firmly in place. Check the system often for clogging of the filter: look for surging within the AA II system. Change the filter when necessary as follows: run DDW

through the filter to get a baseline. Disconnect the sample line from the bottom of the filter and place it in DDW. Change the filter with forceps. After the new filter has been flushed with DDW for a minute, reconnect the sample line. Establish a new baseline, then connect the inflow to lake water.

If mapping is to be done in an area with unusual nutrient concentrations, change the standard calibration on the colorimeters accordingly. Example: When mapping the Grand River plume the std. cal. of the chloride colorimeter is halved, otherwise the high concentrations will be off scale.

Carefully record all mapping events on both the chart paper and a separate log. Record the ship time of DDW rinses, standard solutions, lake water, turns, stops, etc.

Troubleshooting

Problems Manifest on the Strip Chart and Their Possible Causes:
Too much drift - either or both phototubes bad, old reagents.
No peaks - bad reagent, recorder fuse blown, reagent not pumping.
Noise but peaks - system not cleaned well, bad reagents, small bubble trapped in flow cell, particulate matter in the system.
Excessive noise - bubbles in flow cell.
Pen off scale - bubbles, bad or exhausted reagent.

Plumbing Problems: Unwanted bubbles enter system when a reagent is exhausted, when the sampler misfires, or when a plumbing leak develops.

Chemical Problems: Reagents must be made up and stored properly. Reagents that are most likely to go bad with age or improper storage include: all NH_3 reagents, PO_4-molybdate and ascorbic acid reagents, SiO_2-ascorbic acid. However all reagents must be suspect of contamination.

Optical Problems: When necessary replace phototubes and light bulbs, peak the instrument, align the light path, and clean the filters according to the Technicon Manual. (Ideally the colorimeters should be peaked every time the apparatus is moved.)

Electrical Problems: Electronic circuits of both the colorimeters and strip chart recorders are very sensitive. Colorimeters and chart recorders are left on overnight when in daily use. When first turned on they should be warmed up at least one hour before use. The fuses often blow. Baseline noise may be a result of variations in line voltage particularly on shipboard.

Some of the Analyses and Their Individual Problems: Ammonia. The main problems have been with contamination. Follow recommended procedures for keeping sample bottles and system clean. Ammonia can contaminate any open container. Samples should be run immediately. There is no good method for preserving ammonia samples. Standard solutions are suspect for the same reason. Keep air line scrubbed by passage through 5N H_2SO_4 and then DDW.

Nitrate. Color reagent is too old if it has turned pink. Cadmium columns may lose efficiency or cease to work if made improperly or if air is introduced. Compare NO_3

and NO_2 standards to check the efficiency of the cadmium column.

Chloride. Irregular baseline will result if reagents are not filtered properly.

Cruise Preparation and Packing List for Cruises

Reagents: Refer to the following packing list (Table III.2.6.1) to determine how many packages of wet and dry chemicals must be weighed and made up. Code packages with colored tape:

NH_3	red
Cl^3	green
NO_3	yellow
PO_4	blue
Si	pink

and label as to amount and nature of contents. Clean the entire system well. If the system has not been used in the last two weeks run a set of standards to make sure everything is operating properly. Check expendable items such as graph paper, etc. Fill all pens. Make up new stock solutions. Transport stock solutions and liquid reagents on ice in a cooler.

Packing List for Cruises:

Main Parts

- sampler
- pumps-manifolds
- colorimeters (5)
- voltage stabilizers (5)
- plug strip
- chart recorders (2 dual pen, 1 single pen)

Accessories

- inline filter and plumbing
- waste-jugs and plumbing

Tools

- screw drivers (small, large)
- hemostats (2 or 3)
- scissors
- brown AA parts box
- forceps
- c-clamps (10)
- large paper clamps
- copies of methods and manuals
- filament tape
- pen cleaner (syringe with fine tygon tubing attached)

Supplies

- chart paper (2 kinds)
- ink and pens
- graph paper
- marking pens
- Kimwipes
- paper towels
- aluminum foil
- rinsed filters
- standard recording sheets, data sheets
- Propipets
- red, blue, yellow, pink, and green tape
- glass wool (for NO_3 - Cd column)
- glass tubing (for NO_3 - Cd column)

Spare Parts

- pump tubes (2 entire sets)
- transmission tubing
- AA plumbing connectors, T's (mostly stored in brown AAII parts box)
- spare coils, glass plumbing
- light bulbs for colorimeters
- phototubes
- flow cells
- fuses for recorders, pumps, samplers and colorimeters

TABLE III.2.6.1. Guidelines for estimating preweighed quantities of Auto-Analyzer reagents for use on a research cruise.

Analysis	Reagent	Components	Amount required per 4 hr run	12 hr run
NH_3	Alkaline Phenol	17.5 mL Phenol	17.5 mL	17.5 mL
		12.0 g NaOH	1 vial	1 vial
	EDTA-Nitroprusside	1.86 g EDTA	1 vial	1 vial
		0.125 g Sodium Nitro-ferricyanide	1 vial	1 vial
	Sodium Hypochlorite	15 mL Chlorox	15 mL	15 mL
NO_3	Ammonium Chloride	10 g NH_4Cl	1 vial	2 vial
	Color Reagent	*	80 mL	240 mL
Cl	Mercuric Thiocyanate	*	100 mL	300 mL
	Ferric Ammonium Sulfate	*	100 mL	300 mL
SiO_2	Ammonium Molybdate	10 g $(NH_4)_6Mo_7O_{24}\cdot 4H_2O$ 0.1N H_2SO_4 (make up 1000 mL)	200 mL	600 mL
	Oxalic Acid	50 g $H_2C_2O_4\cdot 2H_2O$	150 mL	450 mL
	Ascorbic Acid	4.4 g $C_6H_8O_6$	1 vial	1 vial
PO_4	Ammonium Molybdate Working Reagent	150 mL 4.9N H_2SO_4	60 mL	170 mL
		45 mL Ammonium Molybdate solution		
		15 mL Antimony Potassium Tartrate solution		
	Ascorbic Acid	2.0 g $C_6H_8O_6$	1 vial	1 vial

All dry chemicals pre-weighed and placed in polyethylene fliptop vials. Liquid chemicals are poured from onboard stocks.

*Bring sufficient volumes of these liquid reagents to last the entire cruise.

Glassware, Plasticware
(minimum needs)

- 1, 2, 3, 4, 5, and 10 mL Class A volumetric pipets (3 sets) for making standards
- 10 mL volumetric pipets (2) for sample dilutions
- 5 mL and 10 mL graduated pipets
- 100 mL volumetric flasks (10 for standards, 2 for dilution, 1 for PO_4 analysis)
- 250 mL volumetric flasks (1 for SiO_2 analysis, 1 for PO_4 analysis)
- 500 mL volumetric flasks (3 for NH_3)
- 1000 mL volumetric flasks (3 for reagents, 1 for standards)
- 25 mL graduated cylinders (1 for NH_3, 1 for SiO_2)
- 100 mL graduated cylinder (for PO_4 analysis)
- 250 mL graduated cylinder (for PO_4 analysis)
- 15, 30, 50, 100, 150, 250, 400 mL beakers (1 of each)
- 50 mL beakers (1 for mixed standards, 1 for NH_3 standards, 1 for PO_4 standards)
- 1000 mL plastic beakers (3 for DDW rinse)
- 2000 mL plastic beakers (2 for soaking filters)
- 4000 mL plastic beaker (for soaking filters)
- 1000 mL brown plastic reagent bottles (2 for SiO_2, 2 for Cl, 2 for NO_3, 3 for HN_3)
- glass petri dish for packing cadmium columns

Accessory Chemical Reagents

- Stock standard solutions
- conc. H_2SO_4 - 50 mL
- acetone - 250 mL
- cadmium - enough for 6 columns
- 2% $CuSO_4$ solution - 200 mL
- 10% HCl - 2 L
- Levor IV - drop bottle
- Brij 35 - drop bottle
- 10% KOH - 150 mL
- DDW ~20 L jug/day
- Chlorox

Pump Tube Delivery Table

From time to time it is necessary to change to slightly larger or smaller pump tube sizes to adjust for variations in flow due to aging of pump tubes or other factors. Remember that the change of one pump tube may necessitate the change of several others. Check by adding up all inflows and outflows and watch the bubble pattern carefully for several minutes after making any changes. Minor imbalances can take hours before manifesting themselves. The following chart is included to aid in the selection of replacement pump tubes should a change become necessary.

TABLE III.2.6.2. Tube sizes and deliveries for standard AutoAnalyzer pump (Source: Technicon Corp.).

Tube I.D.	Shoulder Colors	Clear Standard Delivery cc/min min	ave	max	Solvaflex Delivery cc/min min	ave	max	Acidflex Delivery cc/min(approx.)
.005	Orange Black	.005	.015	.029	.005	.015	.029	n.a.*
.0075	Orange Red	.016	.03	.048	.016	.03	.048	n.a.*
.010	Orange Blue	.032	.05	.072	.032	.05	.072	n.a.*
.015	Orange Green	.075	.10	.128	.075	.10	.128	n.a.*
.020	Orange Yellow	.13	.16	.19	.13	.16	.19	n.a.*
.025	Orange White	.19	.23	.27	.19	.23	.27	n.a.*
.030	Black	.28	.32	.36	.28	.32	.36	0.34
.035	Orange	.37	.42	.47	.37	.42	.47	0.43
.040	White	.54	.60	.66	.51	.56	.62	0.53
.045	Red	.73	.80	.87	.64	.70	.76	0.64
.051	Grey	0.92	1.00	1.08	0.81	0.88	0.93	0.78
.056	Yellow	1.12	1.20	1.28	0.99	1.06	1.13	0.92
.060	Yellow Blue	1.31	1.40	1.49	1.14	1.21	1.29	1.06
.065	Blue	1.50	1.60	1.70	1.29	1.37	1.45	1.19
.073	Green	1.90	2.00	2.10	1.60	1.69	1.78	1.44
.081	Purple	2.37	2.50	2.63	1.92	2.02	2.12	1.71
.090	Purple Black	2.77	2.90	3.03	2.31	2.42	2.53	2.03
.100	Purple Orange	3.26	3.40	3.54	2.77	2.89	3.01	2.39
.110	Purple White	3.75	3.90	4.05	3.26	3.39	3.52	2.76

*Not available.

Introduction

Phosphorus frequently limits plant growth in fresh water environments. Increased phosphorus is the single most important factor in cultural eutrophication of lakes and rivers in general (Schindler 1974, 1977), and the Laurentian Great Lakes in particular (Schelske 1975). Phosphorus is present in a variety of forms in lake water, and it is quickly cycled between forms. Because of this total dissolved phosphorus (TDP), or more usually total phosphorus (TP), is often used as an overall measure of the status of a lake relative to phosphorus eutrophication.

Principle of the Method

In this method polyphosphates are hydrolyzed and organicallybound phosphorus is oxidized to orthophosphate by digestion with persulfate, which decomposes upon heating to form hydrogen peroxide, the oxidizing agent, and sulfuric acid (Menzel and Corwin 1965). The liberated phosphorus combined with the originally present orthophosphate are measured colorimetrically by an automated version of the Murphy and Riley (1962) method using a Technicon AutoAnalyzer I connected to a Gilson Sampler. The soluble fraction is separated from the total fraction by filtration through a 0.45µ Millipore filter. Samples for TDP and TP are taken from the same Niskin bottle water sample, and are digested, and run concurrently. Values for particulate phosphorus can be calculated by subtracting the TDP concentration from the TP concentration.

Sampling and Storage

Total phosphorus samples are taken directly from the Niskin bottle. Soluble phosphorus samples are filtered through a presoaked 0.45µ Millipore filter. Both filtered and unfiltered samples are stored in 2-oz polyethylene bottles, frozen at -10°C.

Upon thawing for analysis the samples should be processed as soon as possible to minimize loss due to adsorption to the container walls. Before the sample is thawed 0.1 mL (1 drop) of concentrated sulfuric acid is added to each bottle. Upon thawing the samples are shaken vigorously and immediately transferred to glass test tubes for concentration by evaporation and digestion.

Equipment and Supplies

AutoAnalyzer I (Technicon)
automatic sampler (Gilson)
membrane filters - 0.45 µ
  (Millipore HA)
polyethylene bottles - 2 oz
  (Nalgene)
test tubes - 17 mm x 150 mm
  (Pyrex); calibrated at 15 and
  25 mL using volumetric pipets
test tube racks - 22 mm hole,
  galvanized
Repipet - 10 mL
constant temperature oven
hot plate (optional)
Vortex mixer
Parafilm
aluminum foil

Reagents

5% Persulfate Solution: Dissolve 100 g of potassium persulfate ($K_2S_2O_8$) or 85 g of ammonium

persulfate [$(NH_4)_2S_2O_8$] in 1800 mL of distilled deionized water (DDW) in a 2000 mL volumetric flask. Dilute to volume with DDW. Both solutions are approximately 3.55% persulfate ion. Do not use both $K_2S_2O_8$ and $(NH_4)_2S_2O_8$ in the same solution; use one or the other for an entire cruise or project. Prepare the solution just prior to its use and discard any undigested solution if it is stored for longer than two hours. Ammonium persulfate dissolves more readily than potassium persulfate, hence is the preferred salt to use. Heat may be used to accelerate solution of potassium persulfate, however the temperature should not exceed 50°C.

Check new lots of persulfate for phosphate contamination against a tested lot currently in use (old lot) by running the following test. Prepare 2 L of 5% persulfate solution using the new lot. If no previously prepared persulfate rinse solution exists, prepare 1 L of 5% solution using the old lot. Boil both solutions for one hour on a hot plate or in the oven. Allow the solutions to cool, dilute to volume and mix well.

In a 100 mL volumetric flask dilute 50 mL of the solution prepared from the new lot to 100 mL with DDW and pour it into clean test tubes. Dilute the old lot 1:1 with DDW and use it as the rinse solution for the sampler.

Run several samples of solution prepared from the new lot through the AutoAnalyzer. If the peaks produced are all two or three units higher than the baseline, the lot should be considered contaminated and discarded. If only a few peaks appear, the source of contamination is the test tubes and not the persulfate.

If the persulfate is satisfactory the bottles from that lot are marked "CHECKED" and dated and the rest of the boiled new lot persulfate may be diluted 1:1 with DDW and used as AutoAnalyzer rinse solution.

Ascorbic Acid Reagent: In a 250 mL volumetric flask, dissolve 4.25 g of U.S.P. quality ascorbic acid in DDW. Dilute to volume. The solution is reported to be stable for one week at 4°C. For best results prepare it fresh daily.

Sulfuric Acid Solution (5.0N): In a 2000 mL volumetric flask, slowly add 280 mL concentrated analytical reagent grade sulfuric acid (H_2SO_4) to 1500 mL DDW. Allow it to cool, then dilute to volume with DDW. Store in a 2 L bottle. The solution is stable indefinitely.

Antimony Potassium Tartrate Solution: In a 250 volumetric flask dissolve 0.6857 g analytical reagent grade antimony potassium tartrate [$K(SbO)C_4H_4O_6$ $1/2H_2O$] in 200 mL DDW. Mix and dilute to volume with DDW. Store in a dark glass bottle in the refrigerator. This solution is stable for many months.

Ammonium Molybdate Solution: In a 1000 mL volumetric flask dissolve 40.0 g analytical reagent grade ammonium molybdate [$(NH_4)_6Mo_7O_{24}$ $4H_2O$] in 900 mL DDW. Mix and dilute to volume. Store in a dark plastic bottle in the refrigerator. This solution is stable until a precipitate forms.

Combined Reagent: Prepare daily, preferably with reagents that have been warmed to room termperature. In a 500 mL volumetric flask combine the following reagents in order and dilute to 500 mL with DDW. Mix after the addition of each reagent.

Combined Reagent:

Sulfuric acid solution	175 mL
Ammonium molybdate solution	56 mL
Antimony potassium tartrate solution	19 mL

Any turbidity can be eliminated by continued mixing. The solution is stable for roughly three days.

Standards

Stock Solution (200 mg PO_4-P/L): Dissolve 0.8789 g of pre-dried (105°C for one hour) primary standard potassium dihydrogen phosphate (KH_2PO_4) in 1000.0 mL DDW. Store in a dark bottle with 1 mL chloroform. Refrigerate. The solution is stable for several months.

Intermediate Stock Solution (2 mg PO_4-P/L): Dilute 10.0 mL of stock solution to 1000.0 mL with DDW. Store in a dark bottle with 1 mL chloroform. Refrigerate. The solution may be stable for many weeks but, for best results, should be made fresh about once every ten days.

Daily Stock Solution (0.2 mg PO_4-P/L): Dilute 10.0 mL of stock solution to 100.0 mL with DDW. Prepare daily.

Working Standards (2.0-20.0 µg PO_4-P/L): Dilute the following five volumes of daily stock solution to 100.0 mL with DDW.

Working Standards

mL Daily Stock solution	µg PO_4-P/L
2.0	4.0
4.0	8.0
6.0	12.0
8.0	16.0
10.0	20.0

Pour standards immediately into clean test tubes, 25 mL per tube, resulting in four tubes of each concentration. Also, prepare in the same manner four blanks using DDW. The blanks are used as a check on the test tubes for phosphate contamination. Evaporate standards and blanks at the same time as the samples are evaporated.

This standard range is optimum for routine Great Lakes samples run at the X10 setting on the colorimeter.

Procedures

Evaporation and Concentration of Samples: Pour 25 mL of sample into calibrated 17x50 mm test tubes and place the tubes in a rack. Loosely cover the rack with a sheet of aluminum foil to reduce phosphate contamination from dust. Evaporate the samples to about 4 mL in a constant temperature oven set at 100°C. Evaporation requires several days due to small surface area of the tubes. Although accidental evaporation to dryness does not seem to have a significant effect on phosphorus concentrations, it should be avoided. If evaporation to dryness does occur, add 3 or 4 mL DDW when tubes are removed from the oven.

Digestion and Dilution: After evaporation, add to all the test tubes 7.5 mL of 5% persulfate solution using a Repipet and mix with a Vortex mixer. Heat the samples at 120°C for ammonium persulfate, 105°C for potassium persulfate, for 1.5 hours in a constant temperature oven. Cool the digested samples, dilute with DDW to 15 mL, and cover with Parafilm (Note 2). Invert twice, stir on a Vortex mixer, and allow to settle for at least four hours before analysis. Parafilm caps are removed just prior to sample analysis.

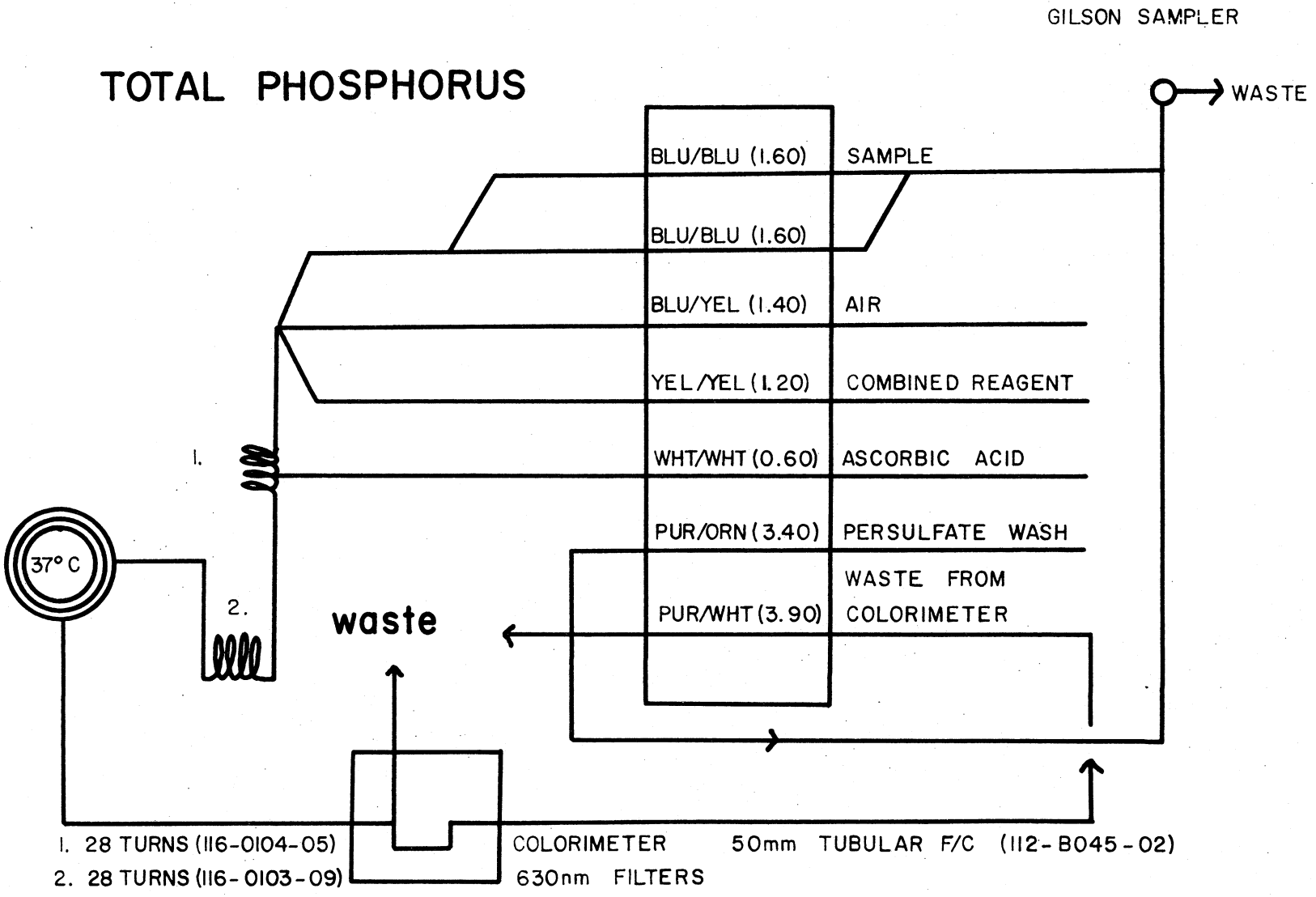

FIG. III.3.1.1 Total phosphorus manifold for AutoAnalyzer II system.

Analysis: Run the standards and samples after digestion and dilution at 30 samples per hour using the AutoAnalyzer I manifold as shown in Figure III.3.1.1. Warm up the AutoAnalyzer and run reagents through the system for one hour before starting the standards and samples. Run the test tube blanks, then standards, then samples. To check consistency run occasional blanks and standards during the sample run.

Notes

1. Test tubes are cleaned before each use. Cleaning includes at least: one rinse with 10% HCl, accompanied by scrubbing with a test tube brush, followed by two rinses with DDW. Tubes are then placed upside-down in racks and allowed to air dry. (Do not dry or drain tubes using paper products as they have high phosphate content.) Before samples are thawed, tubes are numbered using a magic marker. Two tubes for each sample number are required and should be numbered with different colors--one for TP and one for TDP. Tubes are then placed upright in racks and covered with large sheets of aluminum foil until samples are ready to be poured. The tubes remain loosely covered with foil (even while in the oven) to exclude dust until Parafilm caps are put on them after digestion.

Following satisfactory sample analysis, the sample numbers are rubbed off with ethanol and the tubes are emptied and soaked in 10% HCl for several hours. Any material which is still adhering to the glass is brushed off, and the test tubes are rinsed thoroughly with DDW.

2. Parafilm must be kept clean and free of dust. When tubes are capped, Parafilm must not be stretched, but crimped tightly over the lip of the tube (stretched Parafilm dries and splits open allowing dust to fall into the samples).

3. Due to the amount of operator handling in the total phosphate procedure, possibilities of contamination are quite high. When sample concentrations are very low (typical Great Lakes samples are in the range < 2 to 10 µg/L P), small amounts of contamination are significant. Therefore, care in maintaining clean, dust free lab procedures and materials cannot be overemphasized.

4. Completeness of digestion, tested by redigesting and reanalyzing samples, has been satisfactory. We have not established the maximum concentration of phosphate which can be digested by a given amount of persulfate. We have worked with samples as high as 350 µg/L of PO_4, and the amount of persulfate used is undoubtedly adequate for much higher concentrations than this.

5. Phosphate absorbs to container walls at room temperature. In order to minimize losses of phosphate during thawing, samples should be completely thawed but still very cold when transferred from the polyethylene sample bottles to the digestion test tubes. The digestion procedure redissolves any phosphates which adsorb onto the test tubes.

6. Because of the presence of small amounts of phosphate in reagent grade persulfate direct use of crystalline persulfate will introduce nonuniform contamination of

samples. Instead, a measured amount of 5% aqueous persulfate solution is used for each digestion. The absorbance due to phosphate in the persulfate is corrected, during the analyses, by using a wash solution of digested persulfate in the same concentration as that contained in the samples.

7. Presumably, phosphate contamination could be introduced from the glass containers during digestion. However, calibration curves of digested samples have been so consistent and reproducible that any such contamination must be negligible. New glassware should, of course, be soaked with 10% HCl before use.

8. Since phosphate determinations are run under highly acidic conditions, there is no need to neutralize the acid produced by the decomposition of the persulfate.

9. After samples have been digested, they are generally stored at room temperature overnight before analysis. Standards should be evaporated and digested at the same time and under the same conditions as the samples. Analysis should be completed within one week of digestion, as concentrations do decrease with time, probably due to formation of PO_4^{3-} complexes. If analysis must be delayed longer, samples and standards should be redigested at 120°C for 1.5 hours.

10. When the digested samples are run on the AutoAnalyzer any particulate matter in the sample will cause a great amount of noise in the peaks. This noise is intensified since phosphate analyses are usually run at a high range expansion. If after digestion the samples are allowed to sit undisturbed in the test tubes at least four hours, the aspirated solution will not contain particles.

11. Since phosphate will, to a certain extent, adsorb to the walls of the tubing in the AutoAnalyzer system, a longer than normal wash time is allowed between samples. Also, plastic tubing should be kept to an absolute minimum.

12. Trouble Shooting

Symptom: Jagged peaks. Some possible causes:
1) Wash persulfate not boiled
2) Insufficient digestion time
3) Insufficient settling time especially for Total P samples
4) Ascorbic acid reagent bad

Symptom: "Shoulder" on peaks. Possible cause:
1) Pump tubes need replacement

Symptom: Excessive baseline drift. (Note: at high range expansion some drift will nearly always occur. This is acceptable if it is linear.) Some possible causes:
1) Reagent mix not stabilized (can take one hour)
2) Insufficient flushing of wash solution reservoir and feed tube
3) Electronic problems--see appropriate manuals

Symptom: Jagged baseline. Some possible causes:
1) Air bubble in flowcell
2) Crystals forming in old stock solutions
3) Ammonium molybdate problem--cause unknown--suspect brand differences ("J.T. Baker" seems to work best)

References

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Introduction

Particulate silica in the aquatic environment is primarily in two forms: suspended sediments and silica frustules of diatoms. In shallow waters and areas of high turbulence suspended sediments may predominate. In the open ocean and open waters of large lakes the vast majority of particulate silica will be in the form of diatom frustules. In the latter instances total particulate silica may be used as a measure of diatom biomass. Total particulate silica can also be used as a tracer for the input of suspended material (both sediments and diatoms) by rivers into the Great Lakes. Also particulate silica measurements in the nearshore zones may be used to indicate the resuspension of sediments by wave action.

Principle of the Method

A particulate sample is collected on cellulose acetate filters and stored in plastic vials. Complete decomposition of particulate silica is achieved by addition of a HNO_3-HF reagent (Langmyhr and Graff 1959). The solution is allowed to stand for two days at room temperature. The hydrofluoric acid reacts with silica forming silicon tetrafluoride which hydrolyses into silicic acid and fluorosilicic acid. The cellulose acetate filters dissolve in the nitric acid. After decomposition, the excess hydrofluoric acid is complexed with boric acid, taken up in water, and the sample determined for silica by atomic absorption spectrometry using a nitrous oxide-acetylene flame (Bernas 1968).

Sampling and Storage

Lake water samples (600 mL) are filtered through 47 mm, 0.45μ HA Millipore filter. The filters are stored in plastic flip-top vials.

Equipment and Supplies

Atomic Absorption Spectrophotometer (Perkin-Elmer Model 290B)
automatic dispenser
beakers (Nalgene)
flip-top vials (Olympic Plastic, Los Angeles)
plastic serilogical pipets - 5 mL, 10 mL
safety glasses and/or face shield
scoop (1/4 teaspoon)
vinyl medical gloves
volumetric flasks - 1000 mL (Nalgene)
hydrofluoric acid clean-up kit

Reagents

48% Hydrofluoric acid
70% Concentrated nitric acid
Boric acid - 99.99+, Certified purity
Silicon dioxide - pure spec (Johnson, Matthey & Co., Ltd.)
Hydrofluoric acid clean-up kit
Nitrous oxide (Grade A)
Acetylene (300 cu ft welding grade)

Preparation of Standards

Weigh a series of at least five silicon dioxide standard samples (10.0-100.0 mg) and place in plastic flip-top vials. Add 4 mL distilled de-ionized water (DDW), 3.0 mL of hydrofluoric acid and 1.5 mL HNO_3. Allow two days for dissolution even though it should occur rapidly.

To each vial add approximately 2.8 g of boric acid (three 1/4 teaspoon scoops) and aid its dissolution by shaking the vial. Wash the contents of the vial into 1000 mL Nalgene volumetric flasks and dilute to volume with DDW.

Procedures

Decomposition of Samples: Add 0.5 mL nitric acid to the sample vial, then add 1.0 mL of hydrofluoric acid. Force the filter down so that it is wetted with the acid mixture. Seal the cap and mix the contents with mild agitation. Allow the sample to stand for at least 48 hours at room temperature. Then using an automatic dispenser add 8.5 mL of DDW, and 0.95 g of boric acid (approx. 1/4 teaspoon). Shake until the boric acid dissolves and allow to stand for about 30 minutes prior to analysis. If an excess of boric acid is added, undissolved crystals will be present. Run blanks (unused filters) (3) with each batch of samples.

Analysis of Samples: Consult the atomic absorption analytical methods manual and operation manual for information on atomic absorption techniques and instrument operation and maintenance. For operation of the Perkin-Elmer 290B use the following settings:

Lamp current	given on lamp
Slit	7 A
Element	145-149
Maximum	all the way counter-clockwise
Damp	2-4
Air pressure	20 psi
Acetylene pressure	6 psi
Nitrous oxide pressure	30 psi
Air flow	14.4
Acetylene flow	14.0
Nitrous oxide flow	11.0

Before igniting the flame check the following:

Safety glasses and/or face shield are on.
Nitrous oxide burner head is in place.
There is water in the loop of the waste drainage tube and the end of the tube is submerged in 4-5 inches of liquid. If the drainage tube is opened to the air, there will likely be a flash back (explosion).
The nitrous oxide pressure regulator heater is on and the Variac setting is at 115.
The lamp output is stable -- this requires a warm-up time of 4 hrs.
There is over 100 psi in the acetylene tank. The tank should be replaced at 75 psi.
There is over 150 psi in the nitrous oxide tank.
The lamp is aligned.
The burner head is aligned.
The burner head is clean.
The nebulizer is adjusted.

Proceed by maximizing the wave length with the fine select knob. Turn on the air, light match, turn on the acetylene, and ignite the flame. Change the acetylene fuel flow from 14.0 to 11.0. Turn on the N_2O. Adjust the fuel flow while the instrument is aspirating H_2O to give a red (pinkish) flame.

To run samples set the recorder pen on the 10.0 chart division and wait for a stable baseline (in reality it may drift slightly). Run at least five standards, 10-15 samples (break samples between stations), three standards, etc., and at least five standards at the end. After the first run of standards check the sensitivity against past runs (consult standard curve records). If the sensitivity is

significantly low (>10%), shut the flame down (see below) and recheck that the burner head is clean and the nebulizer adjusted. Save the last five samples run in the day to be the first ones run the next day to check reproducibility. When aspirating samples, to prevent clogging. keep the end of the tube out of any undissolved boric acid at the bottom of the vial. If the aspirator tube becomes clogged it may be cleared by inserting a thin wire up the tube and through the nebulizer.

To shut down the atomic absorption spectrophotometer turn the fuel flow dial to 11.0. Flip back to air. Listen for a change in pressure and then quickly turn the fuel flow dial to 14.0. Flip down the fuel toggle switch. Shut off the gas tanks. Bleed the acetylene line with the air still flowing over the burner head. Then bleed the N_2O line. DO NOT BLEED THE ACETYLENE AND N_2O LINES TOGETHER. THE TWO GASES FORM AN EXPLOSIVE MIXTURE WHICH CAN BE IGNITED BY A HOT BURNER HEAD.

Records and Calculations

After each run plot a calibration curve of the standards run at the beginning and end of the run in the standard curve book (Figure III.3.2.1). Record all data in the Particulate Silica Form (Figure III.3.2.2).

Samples are concentrated 60 fold. That is, 600 mL of lake water is concentrated into a 10 mL sample. To find the concentration of the actual lake water from this concentrated sample, one would normally divide the concentration of the concentrated sample by 60. To eliminate this calculation we divide our standards by 60 when running against the concentrated samples. This gives the volume-corrected concentration of the lake water.

Notes

1. Hydrofluoric acid and its vapors cause severe burns which may not be immediately painful or visible. When working with hydrofluoric acid wear protective garments, safety glasses and vinyl gloves. Avoid breathing the vapors. If any part of your body comes in contact with hydrofluoric acid, immediately flush thoroughly with cold water for 15 to 30 minutes and afterward wash with a solution of sodium bicarbonate, followed by soaking the affected area in 10% calcium chloride for an hour. Call a physician. Remove and discard any garment that has come in contact with the acid. If there are any spills use the hydrofluoric acid clean-up kit.

2. Carbon deposits may form on the AA burner head; they can be removed using a metal rod. If a run is in progress always run three standards after removal of carbon from the burner head, clearing a clogged nebulizer, or changing any dial.

References

Bernas, B. 1968. A new method for decomposition and comprehensive analysis of silicates by atomic absorption spectrometry. Anal. Chem. 40: 1682-86.

Langmyhr, F. J. and P. R. Graff. 1959. Studies in the spectrophotometric determination of silicon in materials decomposed by hydrofluoric acid. I. Loss of silicon by decomposition with hydrofluoric acid. Anal. Chimica Acta 21: 334-339.

PROJECT:	ELEMENT: P-S:O_2
DATE:	AMP:
OPERATOR:	WAVELENGTH:
DAMP:	REC. OUTPUT:
SLIT:	REC. SPAN:
FUEL:	CHART SPEED:

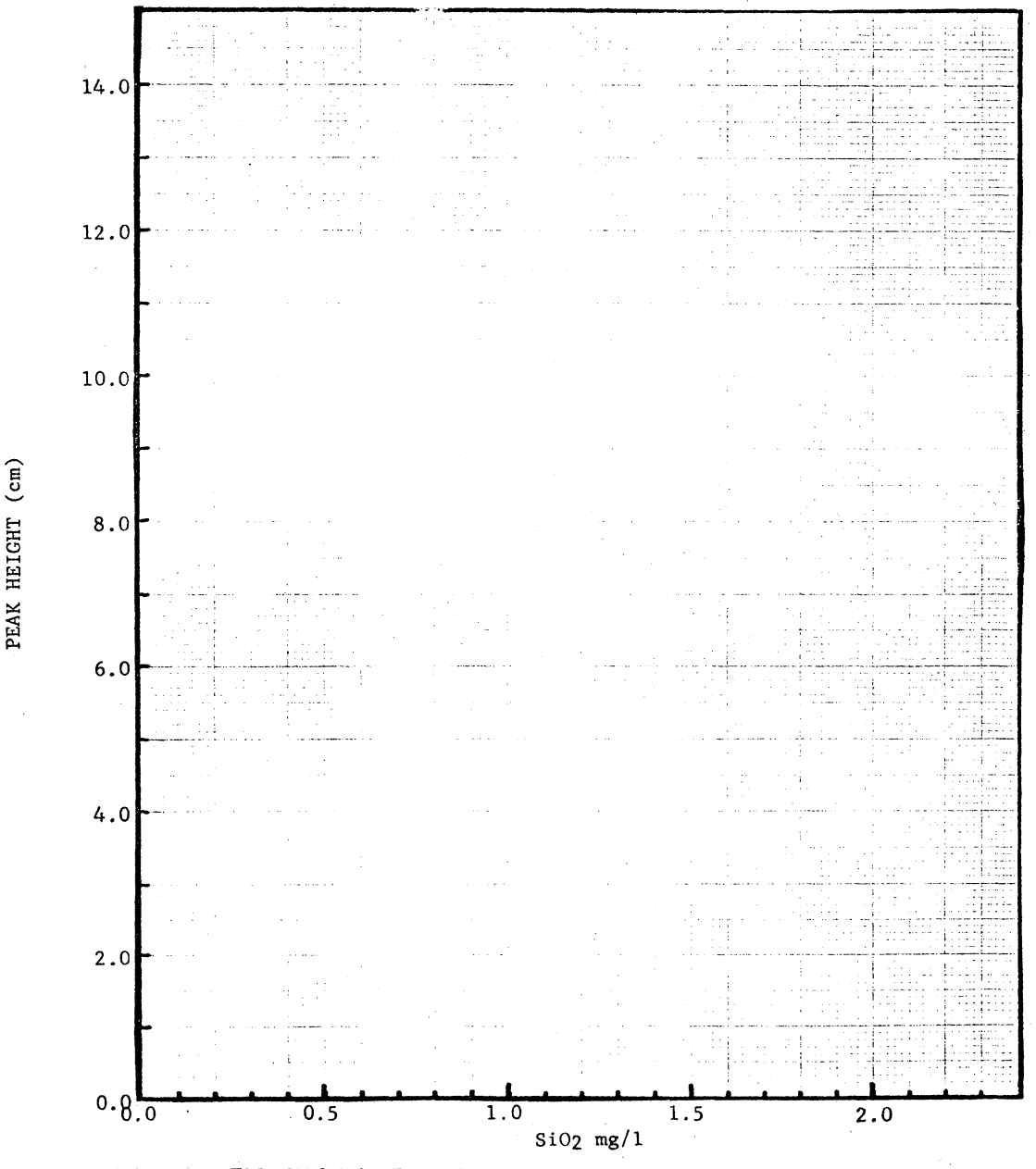


FIG. III.3.2.1 Form for total particulate silica standard curves.

_ _ - _ _ - _ _ _ _ - _ _ _ _ -

_ _ - _ _ _ _ - 0 3 - 1 6

	BOOK	PAGE
*PSIO	_ _ _ _	_ _ _ _

PROJECT:	ELEMENT: P-SiO_2
DATE:	AMP:
OPERATOR:	WAVELENGTH:
DAMP:	REC. OUTPUT:
SLIT:	REC. SPAN:
FUEL:	CHART SPEED:

STANDARDS

STANDARD	READING

CHECKS

CHECK	READING

SAMPLES

SAMPLE	H.	READING

SAMPLES

SAMPLE	H.	READING

FIG. III.3.2.2 Log sheet for total particulate silica data.

Introduction

Carbon and nitrogen are primary components of all living matter. In aquatic systems particulate carbon and nitrogen are a measure of living organic matter and organic detritus. In the open waters of the ocean and large lakes organic detritus is derived from the pelagic food chain. In small lakes and nearshore regions detritus may also be of terrigenous origin as from leaf litter or from the erosion or organic rich soils.

Principle of the Method

The CHN analyzer is an instrument designed to provide a rapid, semi-automatic means for measuring the carbon, hydrogen, and nitrogen content of materials. The sample is converted to its component parts in the form of N_2, CO_2, and H_2O in the presence of an oxidant at high temperature.

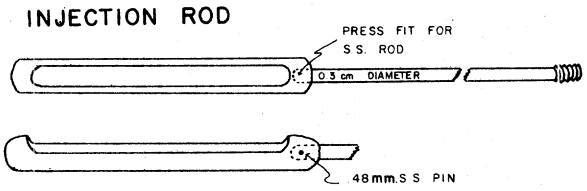
After combustion the components are swept into the chromatographic system by helium carrier gas and separated by their different affinities for the Porapak Q (80-100 mesh) column packing. Peak heights and areas are determined by a thermal conductivity detector as the nitrogen, carbon dioxide, and water peaks emerge from the column. Nitrogen, carbon and hydrogen are determined from the N_2, CO_2, and H_2O peaks respectively by comparison of the unknowns with standards of known chemical composition.

Sampling and Storage

Lake water (1000 mL) is filtered through a previously combusted 25 mm GFC filter. The filters are then folded in half with the sample on the inside, and placed in individual glassine envelopes. The samples are dried in a vacuum desiccator, and stored in a desiccator in the freezer until they are ready to be analyzed.

Equipment and Supplies

Cahn Electrobalance
muffle furnace
Hewlett Packard CHN Analyzer Model 185B
modified sample injection rods (Fig. III.3.3.1)



	FOR 2.5cm GF/C FILTER	FOR 47mm GF/C FILTER
OVERALL LENGTH	36.8 cm	36.8 cm
THREAD LENGTH	2.5 cm	2.5 cm
HEAD LENGTH	3.8 cm	5.9 cm
HEAD DIAMETER	0.26" OR 0.66 cm	0.26" OR 0.66 cm
CHAMBER LENGTH	3.0 cm	4.3 cm
CHAMBER WIDTH	0.5 cm	0.5 cm
CHAMBER DEPTH	0.4 cm	0.3 cm
CHAMBER DEPRESSION	0.1 cm	0.1 cm

FIG. III.3.3.1 Injection rod for combustion furnace.

sample boats. Sample boats are cut out from aluminum pans to fit the modified sample injection rods. The boats are cleaned with acetone and distilled water and are dried in the muffle furnace at 450°C before

using.

pre-combusted filters. Whatman GF/C (2.5 cm) filters are wrapped in aluminum foils and combusted in a muffle furnace at 450°C for one hour.

2-stage pressure regulator for helium carrier gas

glassine envelopes (for 2x2 photographic negatives)

The standards, boats, and ashed filters are kept in desiccators when not in use to prevent adsorption of moisture and other contamination from the surroundings.

Reagents

Catalyst: an oxidizing catalyst conditioned at 250°-275°C for a minimum of 16 hours before using (purchased from Hewlett-Packard).

Helium Gas

Standards

There are three standards of different CHN composition available from Hewlett Packard: Cystine, acetanilide, and cyclohexanane-2,4-dinitrophenyl hydrazone. Cystine contains 29.99% C, 5.03% H, 11.66% N; acetanilide contains 71.09% C, 6.71% H, 10.36% N; and cyclohexanane-2,4-dinitrophenyl hydrazone contains 51.79% C, 5.07% H, 20.14% N.

Procedures

Preparation of Standards: Open lake water samples typically have a CHN composition similar to cystine and, therefore, cystine is typically used for standards. Weigh out five cystine standards on combusted Whatman GF/C filters using the Cahn electrobalance. The weights of the standards should be in the range 0.8 to 3 mg. Add a moderate amount of catalyst (enough to cover the amount of standards on the filter) to each standard. Then roll the filter paper with contents between two small glass capillaries so that it would fit in the sample holder. Place a boat on the sample rod and then insert the rolled filter. Care should be taken to avoid spillage during the entire process.

Preparation of Samples: Remove the filter from the glassine envelope. Add sufficient catalyst to cover the residue on the filter, then roll the filter and contents in the same manner as the standards.

Analysis of Standards and Samples Using CHN Analyzer: Turn on the CHN Analyzer and allow it to warm up for approximately six hours. When ready, the temperature of the reduction furnace should read between 400-500°C, oxidation furnace 1100°C, column oven 100°C, and oven shell 20°C less than the oven temperature. Set the combustion cycle at 20 seconds. Pressurize the system for about ten minutes by closing the carrier flow valve, then zero the baseline of the chart recorder before proceeding.

Before any standards or samples are run, insert the sample rods to be used in the combustion chamber and run through the combustion cycle to burn off any contamination from the air or other sources. Continue running blanks until no peaks are obtained on the chart. Run standards at the beginning and at the end of each sample run consisting of 15-20 samples. Always include all samples taken from the same station in a single run.

Filter blanks, precombusted filters with catalyst, should be run with each new batch of filters, and periodically, to check that there is no contamination from the filters.

Calculations

Using cystine as a standard, compute constants or K factors as follows:

$$K_c = \frac{29.99 \times \text{mg cystine}}{\text{Carbon peak area}} \quad (1)$$

$$K_h = \frac{5.03 \times \text{mg cystine}}{\text{Hydrogen peak area}} \quad (2)$$

$$K_n = \frac{11.66 \times \text{mg cystine}}{\text{Nitrogen peak area}} \quad (3)$$

CHN percentages of the samples are then calculated using the following formulas:

$$\%C = K_c \times \frac{\text{Carbon peak area}}{\text{Sample weight}} \quad (4)$$

$$\%H = K_h \times \frac{\text{Hydrogen peak area}}{\text{Sample weight}} \quad (5)$$

$$\%N = K_n \times \frac{\text{Nitrogen peak area}}{\text{Sample weight}} \quad (6)$$

If catalyst or filter blanks are used, they must be subtracted from the peak area before making the above calculations.

Note

1. Particulate inorganic carbon can be dissolved and removed from a sample by soaking the residue on the filter with 0.3% (v/v) H_2SO_4 (Anon. 1975). The remaining residue is then dried and analyzed to determine organic carbon and organic nitrogen.

References

Anon. 1975. Organic carbon and organic nitrogen: particulate. Analytical Methods Manual, Inland Waters Directorate, Water Quality Branch, Ottawa, Canada.

Hewlett-Packard: Instruction Manual for Model 185 CHN Analyzer

Philbert, F. J. and W. J. Traversy. 1973. Methods of sample treatment and analysis of Great Lakes water and precipitation samples, pp. 294-308. In Proc. 16th Conf. Great Lakes Res., Internat. Assoc. Great Lakes Res.

Introduction

Chlorophyll is used as a quick measure of phytoplankton biomass in lake water. The two commonly used methods are the spectrophotometric and fluorometric methods (Strickland and Parsons 1972). At GLRD we use the fluorometric method. This method is not as precise as the spectrophotometric method, but is faster, and requires a smaller sample volume for a given sensitivity. Only chlorophyll *a* is determined, however the sample is acidified and the fluorescence remeasured to correct for phaeopigments. The limit of detection depends on the volume of water filtered and the sensitivity of the fluorometer. With a 250 mL sample 0.1 mg chlorophyll *a*/m^3 can be detected. The 95% confidence limits for a single determination is about ± 8% on values exceeding 0.5 mg chlorophyll *a*/^{3} (Strickland and Parsons 1972).

Equipment and Supplies

Turner Model 111 fluorometer
fluorometer cuvettes
cuvette stand
Vortex mixer
5% HCl in dropper bottle
Parafilm
Kimwipes
pipets
clinical centrifuge
15 mL pyrex graduated centrifuge tubes, with caps
47 mm HA Millipore filters
Millipore filtration apparatus
forceps
250 ml graduated cylinder
90% acetone buffered with $MgCO_3$
amber vials, 5 dram with labels
Repipet

Pre-cruise Preparation

One or two days prior to the cruise departure amber vials are prepared for chlorophyll sample collection. The first step is the preparation of 90% acetone which is used as the extraction solvent for chlorophyll. Since the fluorescence characteristics of chlorophyll varies depending on the amount of water present in the acetone, it is important to always follow the same procedure in the preparation of 90% acetone. The procedure used at GLRD is as follows:

1) Pipet 100 mL of distilled-deionized water (DDW) into a one-liter volumetric flask
2) add 0.1 g $MgCO_3$
3) add 900 mL of 100% acetone
4) stopper the flask and shake. The volume will go down.
5) add more 100% acetone to bring the volume up to the 1-L mark
6) repeat 4 and 5 until the volume remains constant.

The $MgCO_3$ buffers the acetone to keep it basic, preventing the breakdown of the chlorophyll to phaeopigments.

The 90% acetone is transferred to a Repipet and stirred with a magnetic stirrer to keep the $MgCO_3$ in suspension while the amber vials are filled. Five-dram amber vials are filled with approximately 8.5 mL of 90% acetone. Filled vials are tightly capped and stored in the freezer to retard evaporation of acetone until needed.

Procedure

In the field a 250 mL sub sample is filtered through a 47 mm HA Millipore filter using a maximum of 10 psi vacuum. Less than 250 mL may be used for inshore samples. The volume used should be recorded on the station log sheet. Filters are placed in the prepared amber vials, shaken vigorously, and stored in the freezer.

After the samples have extracted in the freezer for at least 48 hours the chlorophyll concentration is determined using a Turner 111 fluorometer equipped with a high sensitivity door, R-136 red sensitive photomultiplier, 110-853 lamp, Corning 5-60 primary filter, Corning 2-64 secondary filter, and 1% or 10% neutral density filter. Allow the fluorometer to warm up at least one hour before use.

Eight samples are removed from the freezer and shaken on a Vortex mixer. The samples are then poured into 15 mL centrifuge tubes and the volume is brought up to 10 mL with 90% acetone. The tubes are centrifuged at approximately 2000 rpm for five minutes. Samples are removed from the centrifuge and 5 mL of the supernatant is transferred by pipet to a cuvette, being careful not to stir up the precipitate. Cuvettes are kept in the dark until the sample is placed in the fluorometer to reduce degradation of the chlorophyll by light. Each sample is placed in the fluorometer and a reading is taken choosing the scale which gives a dial reading of greater than 50. (Any dilution is recorded on the log sheet.)

When the scale and reading has been recorded add one drop of 50% HCl to the cuvette (Riemann 1978). The cuvette is then covered with Parafilm and inverted two or three times to mix the acid. By the time all eight samples have been acidified the first sample is completely converted to phaeopigments (reaction is complete in one minute). Readings of the acidified samples are taken in the same manner as the unacidified samples. Readings should be made within five minutes after acidification.

After the readings are recorded the cuvettes are rinsed twice with 100% acetone, twice with DDW, and twice more with 100% acetone to remove any trace of acid. The centrifuge tubes are given the same rinses, using a test tube brush with DDW to remove any precipitate.

Calculations

$$\text{mg Chl}\underline{a}/m^3 = F_d \frac{\tau}{\tau-1} (R_b - R_a) \qquad (1)$$

$$\text{mg Phaeopigment}/m^3 = F_d \frac{\tau}{\tau-1}(\tau R_a - R_b) \qquad (2)$$

where:

F_d is the door factor, R_b is the reading before acidification, R_a is the reading after acidification and τ is the ratio R_b/R_a for an extract of chlorophyll free of phaeopigments. τ used at GLRD was determined by taking approximately 100 unacidified and acidified readings of natural lake water phytoplankton cultures in log phase growth. An average value of 1.98 was obtained which is significantly lower than the value of 2.2 generally used (Strickland and Parsons 1972). Substituting this value into equations 1 and 2:

$$\text{mg Chl}\underline{a}/m^3 = F_d 2.02 (R_b - R_a) \qquad (3)$$

$$\text{mg Phaeopigment}/m^3 = F_d 2.02 (1.98R_a - R_b) \qquad (4)$$

The door factors (F_d) are determined by making serial dilutions of pure chlorophyll a or a known solution of chlorophyll a free of degradation products which has been measured on the spectrophotometer. Read the fluorescence on as many scales as possible for each dilu-

tion. The results are plotted and a linear regression is used to calculate the slopes which represent the door factors.

D is the dilution factor:

$$D = \frac{\text{vol. filtered (mL)}}{25 \times \text{final vol (mL)}} \quad (5)$$

for the standard conditions

$$D = \frac{250}{25 \times 10} = 1 \quad (6)$$

Otherwise D should be calculated and the values obtained for mg Chl a/m^3 and mg phaeopigments/m^3 must be divided by D.

We have found it most useful to present phaeopigment values as the "phaeopigment fraction" which is phaeopigments divided by the total of chlorophyll plus phaeopigments. Therefore, from equations 3 and 4:

phaeopigment fraction =

$$1.02\left(\frac{1.98}{\frac{F_{db}R_b}{F_{da}R_a}}\right) \quad (7)$$

where F_{db} is the door factor for the unacidified reading and F_{da} is the door factor for the acidified reading. If both readings are made on the same scale the door factors cancel.

Notes

1. The fluorescence of chlorophyll a and phaeopigments is a linear function of concentration. There is, however, a point of saturation after which readsorption and secondary emission destroy the linear relationship. The concentration at which this occurs should be determined for each fluorometer by measuring the fluorescence of a serial dilution of chlorophyll a concentrations as described above for the calculation of the door factor. If linearity is unknown, readings above 50 on the 3X scale should not be used; dilute the sample instead.

2. Before running chlorophylls, the fluorometer should be blanked to zero with the dummy cuvette. The filters should be removed and cleaned with lens paper. They must be oriented in the machine so that the writing on the filters is readable. The filters are not uniform and if they are not replaced in the proper way it will change the properties of the machine.

References

Riemann, B. 1978. Carotenoid interference in the spectrophotometric determination of chlorophyll degradation products from natural populations of phytoplankton. Limnol. Oceanogr. 23:1059-1066.

Strickland, J. D. H. and T. R. Parsons. 1972. A practical handbook of seawater analysis. J. Fish. Res. Board Can. 167: 311 pp.

Introduction

The ^{14}C technique is the only suitable method for measuring the productivity of phytoplankton in areas of low productivity such as the open waters of the ocean or large lakes. In this technique a small amount of radioactive carbon (^{14}C) in the form of bicarbonate is added to the sample. The total amount of CO_2 in the sample is measured (Section III.1.2) before incubation and thus the ratio of $^{12}CO_2$ to $^{14}CO_2$ is determined. After incubation for a suitable period of time the amount of ^{14}C incorporated into the phytoplankton is determined. Knowing the $^{12}C/^{14}C$ ratio in the incubation sample allows the calculation of the actual amount of carbon taken up by the phytoplankton during the incubation period.

Equipment and Supplies

liquid scintillation counter
^{14}C bicarbonate ampules
250 mL hard glass reagent bottles (Pyrex)
aluminum foil
2-3 5 mL glass syringes
mechanism for incubation (either incubator box or floats)
47 mm HA Millipore filters
47 mm Millipore filtration apparatus
scintillation vials (glass)
scintillation cocktail and automatic pipet flask
100 mL volumetric flask
Repipet
2 mL soft-glass, color-break ampules

Preparation of ^{14}C Bicarbonate Ampules

Before going into the field, make up the proper strength solution of ^{14}C bicarbonate and seal in glass ampules. The working solution is made as follows:

Use a high specific activity solution of liquid ^{14}C sodium bicarbonate (~40 mCi/m mole) obtained from a supplier.

Dilute the commercial solution to the proper working strength which is usually 1 μCi/mL. A solution of 1 L of doubled distilled water, 1.0 g $NaHCO_3$, and a few drops of 10% HCl to adjust the pH to about 8.8 is used for the dilution.

Place the solution containing the ^{14}C in a Repipet reserved specifically for use with radioisotopes. Dispense exactly 2.00 mL of solution into 2 mL soft-glass, color-break ampules. Seal the ampules quickly with a natural gas torch.

After cooling, place the ampules in a beaker of tap water with a few drops of phenophthalein. Ann Arbor tap water is normally basic, so the dye colors the water bright pink. Autoclave the ampules 20 min. at 17 psi. Phenophthalein is a particularly effective dye to identify improperly sealed ampules as the basic solution inside the ampule turns pink if even the slightest amount of dye leaks in; discard those ampules which leak. Randomly select 5-10 ampules per lot to be used for standardization. Pack the remaining ampules into the boxes which initially contained the empty ampules.

Standardize the radioactive solution by transferring the contents of one ampule into a 100 mL volumetric flask reserved specifically for use with ^{14}C. Dilute the ampule contents to 100 mL with the pH 8.8 dilution solution described above. Shake the flask thoroughly, and place 1.00 mL from the flask into a scintillation vial. Add 10 mL of scintillation cocktail and 1

mL of phenethylamine to the vial. Repeat this step with at least three 1.00 mL aliquots from each flask, and prepare at least three flasks from as many ampules from each lot. Count the standardization samples on the liquid scintillation counter. A computer program on the Alpha 16 is available to convert the counts to disintegrations per minute (dpm's) per ampule. True dpm's per ampule are found by comparison with external standards and correcting for quench by the channels ratio method. Record raw ^{14}C counts and calculated dpm's in ^{14}C data book.

These lab procedures require a moderate amount of set-up, thus preparation of more than one lot at a time is advisable. On person can seal two lots (1000 ampules) per day.

Field Procedure

Collect the sample using a Niskin bottle or other clean, opaque container. Working in subdued light fill three 250 mL bottles per sample (two light and one dark). Using a syringe with a 2" cannula, remove 2 mL of sample from each bottle to accomodate the ^{14}C solution. Break open one ampule per bottle, and inject the contents of the ampule into the sample bottle with a syringe. A Cornwall syringe can be used for the injection. Rinse the ampule at least twice with some of the sample to transfer C-14 remaining in the ampule. Place the stoppers in the incubation bottles, taking care to avoid any bubbles in the bottles, and then tilt the bottles a few times to mix contents. Wrap the dark bottle in foil and incubate all the samples for the desired length of time (usually four hours). Extreme care must be taken not to expose phytoplankton in the bottles to full sunlight.

After incubation, quickly filter the entire contents of bottle through an HA Millipore filter (maximum of 10 psi vacuum) and rinse the incubation bottle, filtering apparatus, and filter with about 10 mL of distilled water. When sucked dry, transfer the filter to a scintillation vial and add 10 mL of scintillation cocktail using an automatic pipet flask.

In many cases, filling vials with scintillation cocktail the day before the experiments are conducted may be more convenient than filling during the filtration procedure. Care must be exercised that each scintillation vial is numbered on the cap (never mark the glass vial) and each vial number can be identified to the correct sample. Each tray should contain only 98 samples so two empty spaces will serve to separate vials which have filters in them from vials which do not. Store scintillation vials carefully, right side up, for transport back to the laboratory.

Scintillation Counting Procedure

Place the scintillation vials into the scintillation counter, with each vial number matching the number on the counting tray. As each vial is placed in the counter, wipe fingerprints off the glass vial with a Kimwipe. After 98 samples are in the counter, place a blank and a known standard in the counter also. Turn the machine on and allow it to warm-up 24 hours while the samples remain in the dark. Count the samples twice, either for 10 min. or to a preset number of counts, usually 5,000-10,000.

A computer program written for the Michigan Terminal System computer (MTS) converts counts to the desired units -- mg C fixed/ m^3/hr. In that program the raw counts are adjusted to dpm's by subtracting out the blank, correcting to an external standard, and correcting for quenching. To correct for the external standard the formula is: corrected counts = $C(R_s/R_c)$, where R_s = cpm's of standard counted when the currently used lot was standardized, R_c = cpm's (counts per minute) of standard counted with samples, and C = cpm's of the sample. Correction for quenching is complicated and the reader is referred to the Liquid Scintillation Spectrometer Manual for details.

Once true dpm's are found for each sample, ^{14}C uptake can be found by the formula:

$$\text{mg C fixed/m}^3\text{/hr} = \frac{(C \times W \times 1.05)}{A \times H}$$

where C = true dpm's in each sample
W = weight of CO_2 in the original sample as found in the alkalinity calculations
1.05 = an isotope correction factor for the difference in the uptake of ^{14}C to ^{12}C.
A = activity in each ampule for the lot of ^{14}C used
H = hours sample was incubated

Notes

1. Facilities are available to count samples by Geiger counter. To use the Geiger counter, modify the above procedure as follows: after filtration HA filters are attached to aluminum planchets with rubber cement. The planchets are identified by sample location, depth of sample, and incubation bottle number. Store the planchets in a box for return to Ann Arbor. Count the planchets in the planchet counter and convert counts to mg C fixed/m^3/hr by a program written for MTS which specifically handles planchet data.

2. We are dealing with radioisotopes and are strictly accountable for all material, including wastes. All liquid wastes (used dye solution from autoclaving, filtrate from the field) must be placed in special 5-gallon containers available from Radiation Control Services (RCS). All solid wastes (used ampules, paper towels used to mop up spills) go into small drums available from RCS. Used scintillation vials are particularly hazardous from radioactivity, glass, and highly flammable liquids. After no more than 1000 vials have accumulated, they should be boxed and sent to RCS.

3. Any spills exceeding 5 µCi must be reported to your supervisor and possibly to RCS and any spills exceeding 250 µCi must be reported to your supervisor and to RCS. This procedure should be followed to insure that spills are handled appropriately to keep radioactivity from accumulating in laboratory areas. RCS regularly monitors laboratories for excess levels of radioactivity so sloppy procedures will soon be discovered.

4. Neither food nor beverages should be handled while working with radioisotopes nor should they be brought into areas reserved for radioisotope use.

Reference

Hall, C. A. S. and R. A. Moll. 1975.

Methods of assessing aquatic primary productivity. pp. 19-52. In H. Lieth and R. H. Whittaker (eds.). Primary Productivity of the Biosphere. Springer-Verlag, New York.

Introduction

Total phytoplankton slides are used for the determination of phytoplankton species composition and abundance. Semi-permanent slides are prepared suitable for quantitative phytoplankton analysis, with minimum manipulation. Problems encountered with prolonged storage of samples in aqueous media are avoided by this procedure.

Principle of the Method

Lake water is fixed with gluteraldehyde, to which ethanol is added. The mixture is filtered through an AA Millipore filter and the filter is mounted on a slide with clove oil.

Sampling and Storage

Lake water samples are usually taken with Niskin bottles. Withdraw a 125 mL sample from the Niskin bottle into a 4-oz polyethylene bottle after first rinsing the bottle with a small amount of sample water. Fix the sample by adding sufficient 50% gluteraldehyde slowly, with continuous mixing, to obtain a final 4% by volume solution. Continue swirling the sample until diffraction lines are no longer visible. The concentration of fixative is not especially critical, but it must be thoroughly mixed with the sample. Allow the samples to fix in the refrigerator in the dark for at least four hours. The samples may be kept for several days in this condition without noticeable deterioration.

Equipment and Supplies

50 mm x 75 mm slides
43 mm x 50 mm #1 cover glasses
large slide boxes to hold slides
diamond tip engraving pencil to mark slides. (It is not suitable to mark slides with felt tip pens or grease pencils since the reagents used in this procedure dissolve most inks.)
graduated cylinders 100, 50, and 25 mL
filtration apparatus (Figure III.4.3.1). The apparatus should have a stopcock so that vacuum can be controlled. The filter support should have a very fine screen or preferably a sintered metal or glass filter disc. If an apparatus with a coarse screen is used the pattern will be impressed on the filter resulting in a preparation with specimens at different optical planes. Such preparations are extremely difficult to use.
25 mm AA (0.8μ) Millipore filters. Either plain or gridded filters may be used.
4-oz Nalgene polyethylene bottles
vacuum pump
forceps

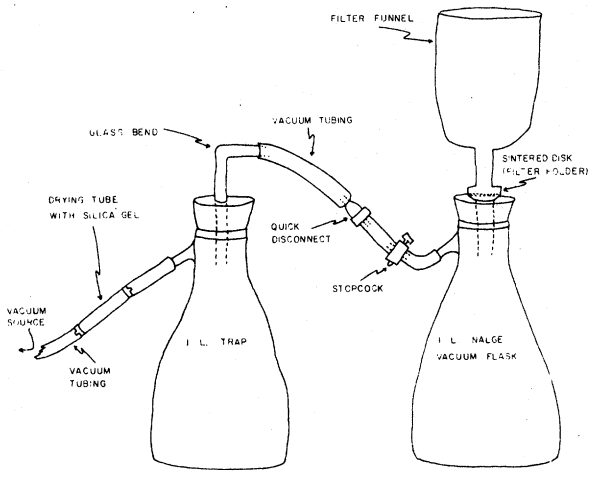


FIG. III.4.3.1 Filtration apparatus.

Reagents

95% Ethanol

50% Ethanol

50% Biological Grade Gluteraldehyde: This reagent should be sealed tightly, stored and transported under refrigeration. If warmed and exposed to light it will polymerize. It can be checked for polymerization by adding five drops of gluteraldehyde to 50 mL of water. If a milky precipitate forms, polymerization has occurred and the gluteraldehyde should be discarded.

Clove Oil

Procedure

After fixation for at least four hours, measure out 50 mL of the sample and slowly add an equal volume of 50% ethanol to the volume of the sample with continuous mixing. Filter the mixture through a 25mm AA Millipore filter using the filtration apparatus described above (Figure III.4.3.1). Filter slowly until the volume left to be filtered is reduced to about 20 mL.

Add 20 mL of 95% ethanol and continue the filtration process. Vacuum should be released slowly just before meniscus reaches the level of the filter. It is important for a good quantitative preparation to reduce the sample volume to an absolute minimum without drawing the meniscus through the filter. If excess water volume is left on the filter some specimens will be lost. If the meniscus is drawn through the filter some of the more delicate forms may be destroyed.

Remove the filter from the filtration apparatus and carefully place it, sample side up, over two to three drops of clove oil centered on a 50 mm x 75 mm slide. Allow the filter to spread out completely on the clearing oil, then immerse the surface with additional clearing oil. Cover the filter with a 43 x 50 mm #1 cover glass, being careful to exclude air bubbles and to center the cover glass. Wipe off excess clearing oil from the surface of the slide and label the slide with an engraving pen. The label should include the project, sample number, volume filtered, station number, date, and depth. Record phytoplankton slides on the log sheets for the stations and depths the samples were collected.

Slides should be placed in a large slide box in a horizontal position. It is essential that the slides remain horizontal during storage and transport (i.e. keep the slide box vertical), otherwise the covers will slip and ruin the preparation.

Slides should be allowed to dry at room temperature for a period of at least four to six weeks. During this period the clearing oil lost though volatilizatin should be periodically replaced. When the slides are dry, seal the edges of the cover glass with hot paraffin.

Note

1. For best results, the slides should be analyzed within a year after preparation. On extended storage the clearing oil will oxidize and become brownish in color. Although the material remains intact, the coloration makes such slides inconvenient to work with.

Introduction

Gross phytoplankton samples are preserved as an archival object record of phytoplankton populations in the Great Lakes. These collections are used in evaluating long-term successional changes in the phytoplankton flora, in resolving certain taxonomic problems, and in standardizing taxonomic interpretations.

Principle of the Method

Lakewater samples are filtered onto a 47 mm AA Millipore filter and preserved with Transeau's solution in 5-dram amber vials sealed in hot paraffin. Samples so treated may be stored indefinitely, and when needed prepared as a permanent slide.

Equipment and Supplies

47 mm Millipore filtering apparatus
5-dram amber screw cap vials
white time tape
Repipet (optional)
47 mm AA (0.8 μ) Millipore filters
200 mL beakers (Pyrex)
Pasteur pipets
hot plate
3" x 1" glass slides
#0 22 mm square cover glasses
hot paraffin

Reagents

Transeau Solution (6:3:1 Preservative): Six parts distilled water, three parts 95% ethanol and one part commercial formalin. The preservative is stable and may be prepared in bulk.

For Cleaning and Slide Preparation:

concentrated H_2SO_4
30% H_2O_2
saturated $K_2Cr_2O_7$
Hyrax

Procedure

Prior to the cruise fill 5-dram amber screw cap vials to the neck with preservative. (This operation is done easily with a Repipet, although the amount of preservative is not particularly critical). Label the vials with standard sequence number labels or with a piece of 3/4 inch white time tape. Pack upright for shipboard use.

On shipboard filter 1 L of raw lake water through a 47 mm AA Millipore filter. Carefully transfer the filter to a preservative-filled vial, reseal the vial tightly, and verify the label. Occasionally samples will be taken where 1 L will not pass through the filter. If less than 1 L is filtered, this must be noted on the vial label, and on the log sheet. These samples will keep for several weeks before being sealed with paraffin.

After returning to the laboratory check the vials to make sure sufficient preservative is present, top up with fresh preservative if necessary, then seal by dipping the cap end of the vials in hot paraffin.

Samples may be stored indefinitely in the paraffin-sealed vials. To prepare a slide for examination of diatoms remove the filter from the vial -- cut it in half and place 1/2 of the filter in a 200 mL Pyrex beaker to dry overnight. Reseal the unused half of the filter in the vial. Dissolve the half filter in a minimum amount (15-20 drops) of concentrated sulfuric acid. Add 50 mL 30% hydrogen peroxide. Slowly add saturated $K_2Cr_2O_7$ solution until a continuous reaction begins (about 2 - 3 mL). Stir briefly. Place the beaker on a hot plate on medium heat (a slow boil) until the reaction is complete (one or two

hours). Let the beakers cool off, then fill them with distilled water. Let the beakers stand for four hours or longer, then decant and refill. Repeat until the solution is colorless and pH is that of distilled water (usually five or six washings). After settling another four hours draw off water until sample is concentrated into less than 5 mL.

Permanent slides are prepared according to Patrick and Reimer (1966) by pipeting a sample of cleaned diatoms onto a clean 22 mm square glass cover slip. The sample is allowed to dry at room temperature, or minimal heat may be applied. When the sample has dried, the coverslip is then heated at a higher temperature to drive off any residual water. A drop of Hyrax is placed on a glass slide and the cover glass inverted onto it. The slide is again heated to evaporate all of the solvent. The cover glass is pressed down, centered, and the slide allowed to cool.

Notes

1. This method provides a rapid and relatively economical process for storage of a large number of samples. Preservation of all phytoplankton forms in each sample is probably not quantitative, and preservation of some forms may not be ideal.

2. When preparing the filters for slides, if the reaction becomes too vigorous remove the beaker from the hot plate and place it in a cold water bath. If the sample evaporates to less than 10 mL add more dichromate solution to the beaker until the reaction is completed.

References

Patrick, R. and C. W. Reimer. 1966. The diatoms of the United States. Vol. I. Acad. Nat. Sci. Philadelphia. Monogr. 13:1-688.

Van Landingham, S. L. 1976. Comparative evaluation of water quality on the St. Joseph River (Michigan and Indiana, U.S.A.) by three methods of algal analysis. Hydrobiologia 48(2): 145-173.